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Experimental and molecular docking study on dna binding interaction of N-phenylbenzohydroxamic acid

Deepesh Khare and Rama Pande*

S.O.S in Chemsirty, Pt. Ravishankar Shukla Univeristy, Raipur, Chhattisgrah, India

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ABSTRACT

The binding interaction of N-Phenylbenzohydroxamic acid (PBHA) with Ct-DNA was measured by four methods, (i) UV absorption, (ii) fluorescence emission, (iii) viscosity and (iv) DNA melting technique. The molecular docking of PBHA with the DNA was performed using 3D-FFT mode and the differences in their binding modes were investigated The result revealed intercalations exist in the system. The values of binding constant, Kb, is $2.01x10^4$ l M^{-1} and Stern Volmer constant K_{sv} is 1.41 $x10^4$ l M^{-1} obtained by UV absorption and fluorescence spectral methods, respectively. The thermodynamic parameters ΔH, ΔS, ΔG were calculated, which suggested that hydrogen bond play major role in the binding of DNA with PBHA. The binding interaction trend is further confirmed by viscosity and DNA melting technique performed on PBHA–Ct-DNA complex which shows the increase in viscosity and DNA melting as a function of PBHA concentration.

Keywords: Hydroxamic acids, Binding constant, Thermal denaturation, Fluorescence, Viscosity.

INTRODUCTION

The studies on molecular interaction between drugs and DNA have great importance to study their biological activity and become an active research area in recent years. DNA is the material of inheritance and control the structure and function of cells. Recently, there has been tremendous interest in studies related to the interaction of small molecules with nucleic acid because of their relevance in the development of new reagents for biotechnology and medicine. These studies are also important to understand the toxicity of drugs containing metal ions [1-3].Ct-DNA is a polymer of alternate sugar phosphate sequence with low protein and high polymerized skeleton. The investigation of drug-DNA interaction is importance for understanding the molecular mechanism of drug action and for the design of specific DNA-targeted drug. The possible interaction model between small molecules and DNA generally follows three models (i) electrostatic binding between cation species and negative charge DNA

phosphate.(ii) groove binding in which molecule bounded in groove of DNA involving hydrogen bonding, Vander Waals interaction force and(iii) intercalative binding involves between drug and base pairs [4-10]. DNA binding is the critical step for DNA activity. To design effective chemotherapeutic agents and better anticancer drugs, it is essential to explore the interactions of drug with DNA.

Hydroxamic acids are drug like molecules as they follow the "lipinski rule of five", with –NOH.C=O- as an active pharmacophore, as a functional group [11]. It forms strong complexes with a range of metals, especially zinc and iron to produce inhibitors of metalloenzymes implicated in the pathophysiology of human diseases. These are generally used as supporting ligands in organometallic chemistry and biology because of their tautomerization and potential as therapeutics agents like inhibitors of enzymes such as peroxidises , ureases, matrix metalloproteinases and hypotensive .[12-16]

Many techniques have been applied for investigation of the interaction of DNA with small molecules including Uv-vis Spectrophotometer, fluorescence, CD spectroscopy, X-ray diffraction, Gel electrophoresis and dynamic viscosity .[17-22] The docking is important in the study of various properties associated with protein-ligand interactions such as binding energy, geometry complementarily, electron distribution, hydrogen bond donor acceptor properties, hydrophobicity and polarizability.[23] In this work, we used UV–vis absorption, fluorescence spectroscopy, viscosity measurements, DNA melting techniques and docking to explore the interaction between PBHA and calf thymus DNA. The knowledge gained from this study should be useful for the development of potential probes for DNA structure and new therapeutic reagents for tumours and other diseases as well as further understanding the pharmacological effects of PBHA.

MATERIALS AND METHODS

1.1. Apparatus

The absorption spectra were measured on Biospectrum BL-198(Elico India) using a 1.0cm quartz cells. The measurement of spectrum was thermostatically controlled by attached temperature controller Gl-635. Fluorescence spectra were performed on Cary eclipsed fluorescence spectrophotometer (Varion, USA) equipped with xenon flash lamp using 1.0 cm quartz cells .pH measurements were carried out with Cyber510 digital pH meter with a combined glass-calomel electrode. The viscosity measurements were carried out using thermo stated Ubbelohole viscometer.

1.2. Reagents

PBHA was prepared by following the reported standard procedure [24].The purity of synthesized compound was ascertained by determining its melting point, and elementary analysis.

Value for M.P, PBHA observed 122^{o}C and reported 122^{o}C .

Elementary analysis C=74.68, N=5.28 and O=15.14.The calculated value are 73.23, 6.57, 15 for C, N and O respectively.

The stock solution of 0.001 M PBHA in DMSO, due to sparingly solubility nature. Ct-DNA (sigma Aldrich Chemical.Co.USA) was dissolved in double distilled deionised water at final concentration 1.55x10^{-4} molL^{-1} and stored at 4^{o}C.

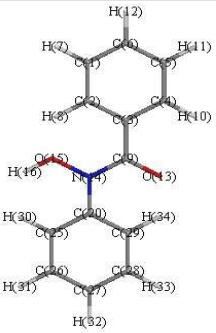

Fig 1: Optimized Geometry structures of PBHA

The concentration determinate by Uv at 260nm using molar absorption coefficient ε_{260}=6600Lmol^{-1}cm^{-1}.Purity of the DNA was checked by absorption ratio $A_{260/280}$ in range 1.8-1.9,which indicates that DNA was sufficiently free from protein[24]. stock solution Ethidium bromide, EB (Sigma Aldrich Chemical.Co.USA)was prepared in tris-HCl and concentration of EB was determined spectrophotometrically at 480 nm using molar absorption coefficient ε_{260}= 5680 mol^{-1} cm^{-1} . pH of buffer solution were adjusted with 0.01M HCl to 7.4, which was prepared by standard procedure. All chemicals were of spectroscopic grade and doubly distilled deionised water was used throughout.

2. Methods

3.1 UV Spectroscopic Method

The UV- spectra of PBHA and DNA-PBHA complex were measured in the wavelength range, 190-380 nm. The competitive interaction was carried out by keeping the fixed amount of PBHA 10 µL and titrated with varying concentration of DNA from 0-126 µL. These solutions were allowed to stand for 8 min to equilibrate. The absorption spectra were then measured at different temperatures 298.15, 303.15 and 313.15 K in the same wavelength range.

3.2 DNA Melting Technique

DNA melting experiment of Ct-DNA and DNA-PBHA complex were performed by monitoring absorbance intensities at different temperature. The absorbance at 260 nm was monitored by 2 °C for solutions of ct-DNA (50 µM) in the absence and presence of the title complex at different concentrations. The absorption data were then plotted as a function of temperature. The melting temperature T_m, which defines as the temperature where half of total base pair is unbounded, was determined as transition midpoint of melting curve. T_m values were calculated by subtracting T_m of the DNA from that of the DNA -PBHA complex.

3.3 Viscosity Measurement

The viscosity experiments conducted at 298.15K on thermostated Ubbelohole viscometer, 10 cm^3 of Tris-HCl was transferred to and flow time was noted,1.55 x10^{-4}mol. L^{-1} Ct-DNA in 10 cm^{3}tris-HCl buffer was then taken in viscometer and flow time read to kept constant DNA concentration. An appropriated amount of PBHA was added at certain r = (HA/DNA) and flow time was measured. The data were presented as $(n/n_o)^{1/3}$ versus r, where n and n_o are viscosity of DNA in presence and absence of PBHA.

3.4 Fluorescence Measurements

Fluorescence emission spectra of PBHA were recorded at 230 to 580 nm upon excitation wavelength at 210 nm using slit width 5 nm each. The fluorescence titration was performed by keeping concentration of PBHA constant (10 µL) and varying DNA concentration 0- 124µL. Ethidium bromide displacement method performed by adding 1µM of EB to each 100 µL of

DNA at λ_{exit} = 475 nm and λ_{em}= 610nm and fluorescence quenching studies were carried out with varying concentration of PBHA. Stern volmer quenching constant K_{sv} is used to evaluate the fluorescence quenching efficiency of PBHA.

3.5 Molecular Docking

Hex calculate DNA-PBHA Docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes . It uses Spherical Polar Fourier (SPF) correlations to accelerate the calculations and its one of the few docking programs. The parameters used for the docking process via HEX docking Software were

- Correlation type – Shape only
- FFT Mode – 3D
- Grid Dimension – 0.6
- Receptor range – 180
- Ligand Range – 180
- Twist range – 360
- Distance Range – 40

RESULTS AND DISCUSSION

4.1 UV absorption measurement is simple but effective method in detecting a small molecule interact with DNA and form a new complex, change in absorbance and position of band should occur. Fig. 2 shows the UV absorption of PBHA and DNA-PBHA complex. In absence of DNA because of π-π* excitation state in PBHA , it showed a significant absorption peak, situated at 234 nm, when bound to DNA intensity sharply decrease with hypochromic shift, which strongly showed binding of PBHA , The intrinsic binding constant of the complex PBHA-DNA was calculated from eq.

$$\mathbf{[CT\text{-}DNA]/(\varepsilon_a - \varepsilon_f) = [CT\text{-}DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)}$$

where, [DNA] is base pair concentration, ε_b , ε_f are apparent absorption coefficients for bounded and free DNA .K_b the ratio of slope to intercept was obtained from a plot of [DNA] / **($\varepsilon_a - \varepsilon_f$)** versus [DNA], a slope 1/ ($\varepsilon_a - \varepsilon_f$) and an intercept 1/ $K_b(\varepsilon_a - \varepsilon_f)$. The value of Intrinsic binding Kb= 2.01 $x10^4$ L M^{-1} is obtained for PBHA.

4.1.1 Thermodynamic Parameters

The acting forces between small molecules and DNA include hydrogen bond, vander waals forces, hydrophobic interaction and electrostatic forces. According to Ross view binding forces is estimated by ΔH and ΔS [25]. The values of ΔH and ΔS are obtained from Van't Hoff equation,

$$\mathbf{Log\ K = -\Delta H/2.303RT + \Delta S/2.303R}$$
$$\mathbf{\Delta G = \Delta H - T\,\Delta S}$$

where, K is the binding constant at corresponding temperature . The value of ΔH,-23.82kJ $mol^{-1}$and ΔS,-16.34 kJ mol^{-1}indicate binding of PBHA with DNA.

The value of ΔG,(-4.82,-4.97and-5.14)10^3kJmol^{-1}forPBHA-DNAcomplex is obtained at 298.15,303.15,313.15K . The negative sign for ΔG revealed that the binding process is spontaneous. While the negative ΔH and ΔS values indicate that hydrogen bond plays a main role in the binding of PBHA to DNA .

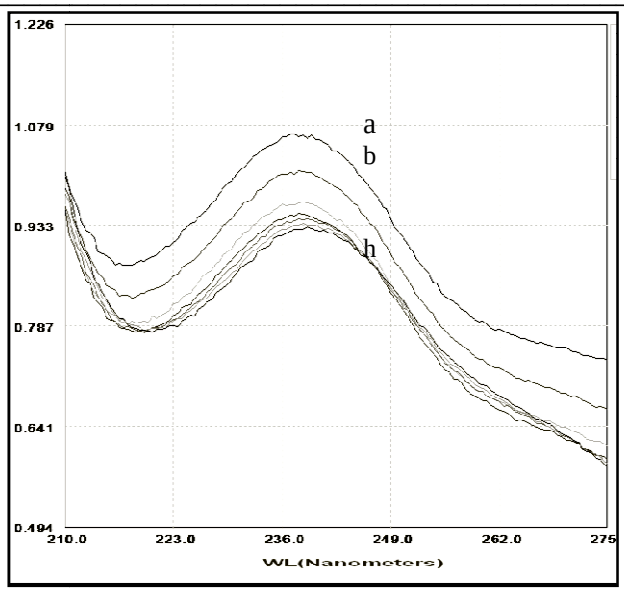

Fig 1: Absorption spectra of the PBHA in Tris-HCl buffer in the absence a and presence of CT DNA (b-h). The PBHA = 10 µL; [DNA] = 0-126 µL.

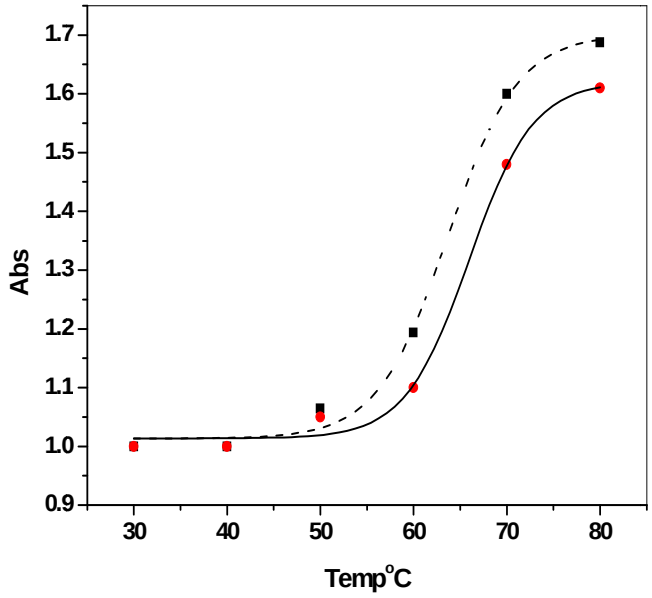

Fig.2.The melting curve of Ct-DNA in absence (--) and presence of PBHA(-)

4.2 **DNA Melting technique**

The double-helical structure of DNA is remarkably stable due to hydrogen bonding and base stacking interaction. On increasing the temperature, the of helix is dissociation to single strand since heat damage the forces. A change in Tm is observed due to interaction of PBHA with DNA. Intercalative binding increases T_m upto 3-8^{o}C. The value of T_m for DNA and PBHA-DNA complex were determined by monitoring the absorbance at 260 nm [26-28]. As can be seen from Fig.3 the T_m of DNA in absence of PBHA is about 64.5^{o}C but the observed Tm of DNA in presence of PBHA is 67.5^{o}C. The interaction of PBHA with DNA cause increase in Tm of PBHA, The result revealed that binding mode of PBHA is intercalation.

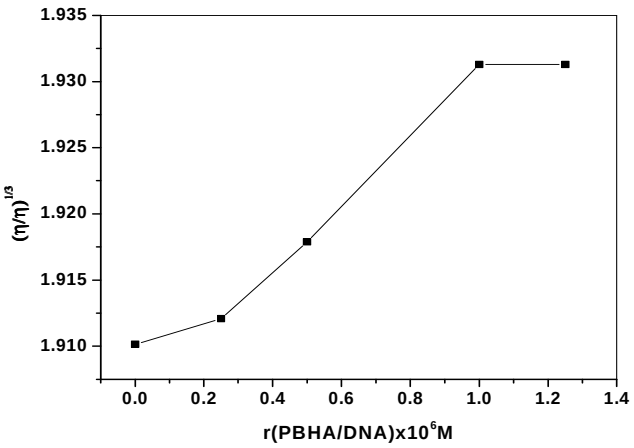

Fig 3: Effect of increasing concentration of PBHA on relatively viscosity of Ct-DNA.

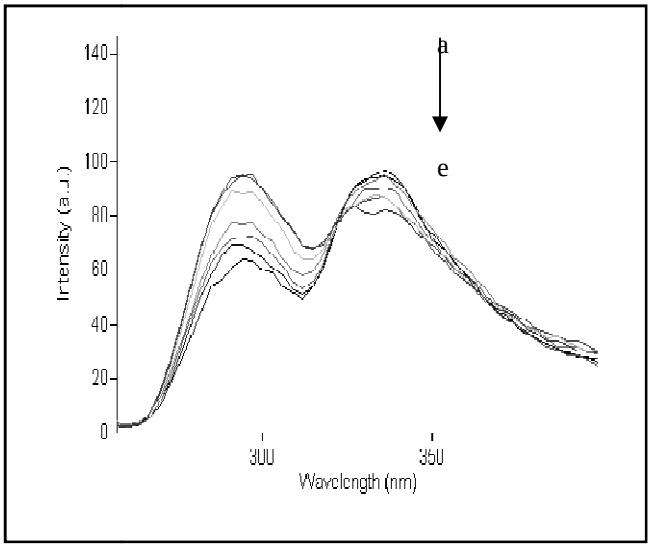

Fig 4: Flurorescence emission spectra of PBHA=10 µL (a) with increasing amount of DNA (b-e)0-120 µL at λ_{exi}=210nm, . λ_{em}=327nm .

4.3 **Visometric Method**

The interaction between PBHA and DNA were further confirmed via viscometric studies. In general, intercalation caused an increase in the viscosity of DNA solution as due to lengthening of DNA helix as the base pair are pushed a apart, It can be seen from Fig 4, a typical relative DNA viscosity increased with increase in PBHA concentration. The information provided is a binding of PBHA to DNA as intercalation.

4.4 Fluoromertic method

PBHA exhibits emission spectra in Tris-HCl at pH 7.4 buffer solution at ambient temperature, with maximum appearing at 276 nm (λ_{ex}= 210nm).The result of fluorescence emission titration for PBHA –DNA at 298 K were illustrated in Fig 4 and5.The fluorescence of PBHA efficiently quenched upon binding to DNA and increasing concentration of DNA resulted decrease in fluorescence intensity of PBHA, which indicated binding interaction with DNA. Quenching is result of variety of molecular interaction included excited-state reaction, ground-state complex formation, collision and energy transfer. Quenching normally refers to nonradioactive energy

from excited species to other molecules. A quantitative estimation of quenching experiments in term of the intrinsic binding constant calculation is obtained from Stern Volmer equation

$$\log (F_o\text{-}F)/F = \log K_b + n \log[Q]$$

where, Kb and n are the binding constant and the number of binding sites, [Q] concentration of DNA, and F_0, and F is the fluorescence intensities in absence and presence of DNA. The linear plot between Fo/F vs DNA and log(Fo-F/F) vs DNA in Fig 6 and 7.The value Kb and n PBHA-DNA complex at 298 K. has been calculated to be 1.41 $x10^4$ lM^{-1} and 1.31, respectively.

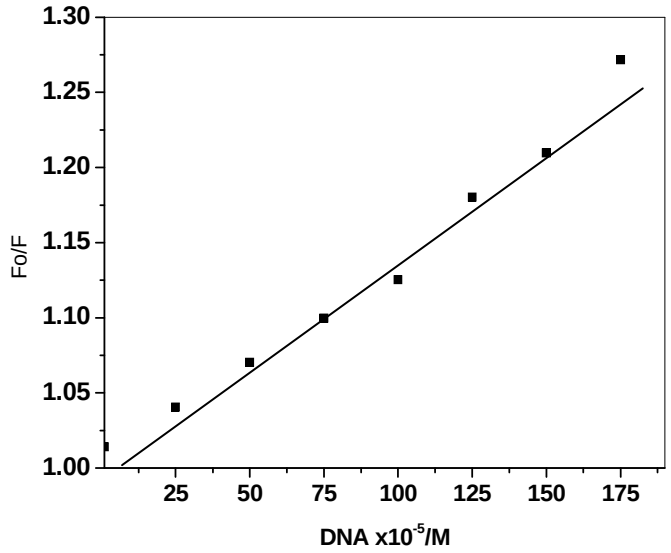

Fig 5: Stern –Volmer plot of PBHA with increasing concentration of Ct-DNA. (0-120 µL) respectively.

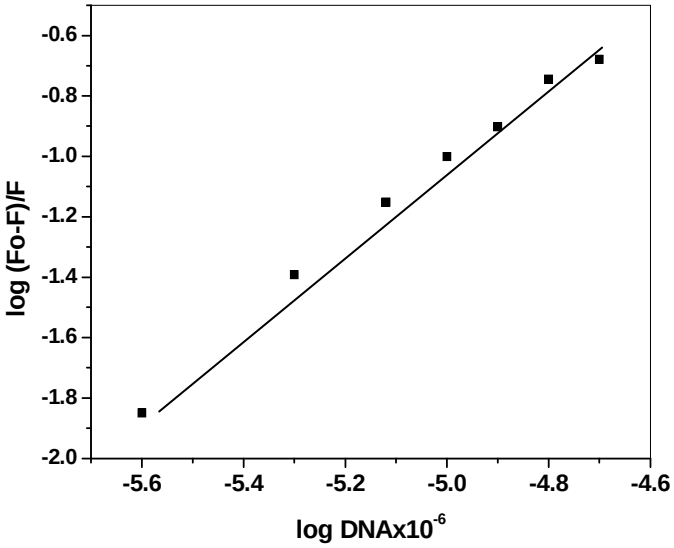

Fig 6: Plot of log (F0–F)/F vs. log [DNA] (0-120 µL), respectively.

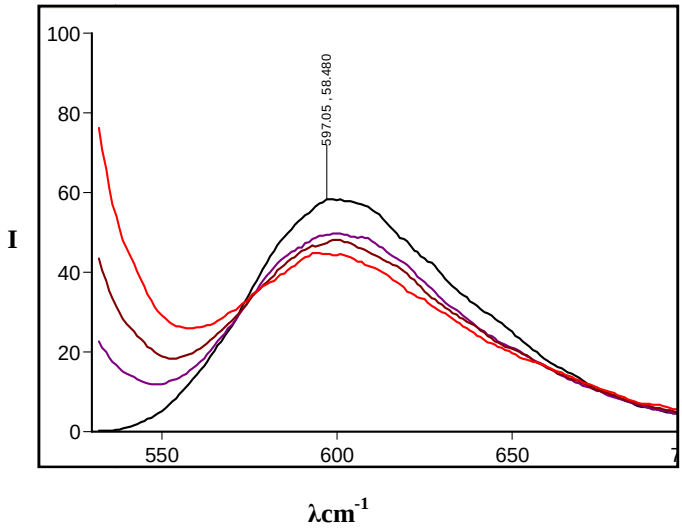

Fig 7. Emission spectra of EB bounded to DNA in absence (-) and presence of PBHA (-)

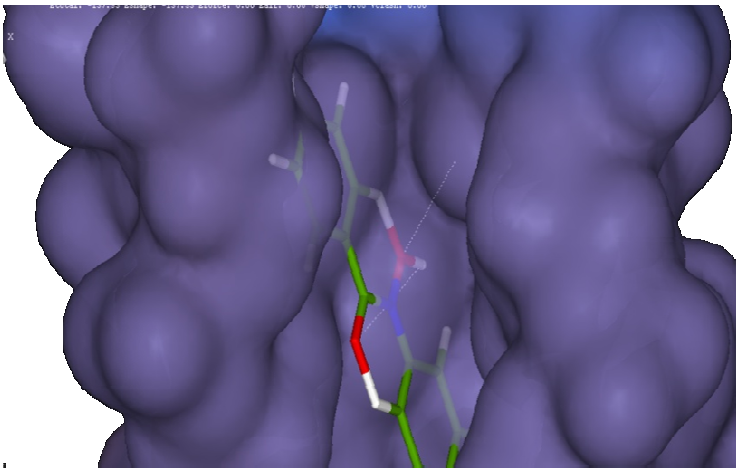

Fig 8. Molecular model of the surface and the active site of the complex of PBHA (stick) and DNA (blue surface).

Beside above experiments, Ethidium bromide displacement method is used to elaborate binding. The fluorescence of EB increases after binding with DNA due to intercalation. Like EB, if PBHA intercalates into the helix of DNA, it would compete with EB for the intercalation sites in DNA, and lead to a significant decrease in the fluorescence intensity of the DNA–EB complex [29-30].Fig.7shows the emission spectra of the DNA–EB complex in the absence and presence of PBHA. With the increased concentration of PBHA added, a remarkable decrease in fluorescence intensity of DNA–EB complex is observed at the maximum of 595 nm. This phenomenon indicates that PBHA substituted for EB in the DNA–EB complex that led to a large decrease in the emission intensity of the DNA–EB complex.

4.5. Molecular Docking

Molecular docking of PBHA on DNA sequences resulted fig.8 in the formation of 3-D docked structures. These structures were studied for various inter-molecular interactions and later subjected to energy analysis of the complexes. The PBHA were docked with the receptor B-DNA using the parameters were above mentioned. The E total energy obtained -137.68 eV for PBHA-DNA Complex.

CONCULSION

PBHA shows one band in its UV spectrum pertaining to π→ π* transition. Upon addition of various concentrations of DNA, hypochromic shift in all UV-spectra were observed. The PBHA–DNA binding constant values conform the strong binding between these PBHA and Ct-DNA, however, Kb value decreases slightly with increase in temperature from 298.15 to 313.15 K due to decreased association between PBHA and DNA. The free energy values for all the compounds are negative showing the spontaneity of PBHA–DNA binding. Intercalative mode of interaction between DNA and small interacting molecule may result due to sharp rise in DNA viscosity .Viscometric studies, therefore could be related to mode of interactions with Ct-DNA. This work, explored the binding interaction and number of binding sites of PBHA with DNA in physiological buffer. It was found that hydrogen bond is responsible for binding of PBHA to DNA. The intercalative binding of PBHA with DNA was deduced by taking account of relevant uv–vis absorption spectra, fluorescence spectra, Ethidium bromide displacement quenching effect, thermal denaturation and viscosity measurements. The docking results revealed that intercalated mechanism is followed by PBHA to bind with Ct-DNA and thus will be very helpful to the design of new drug.

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REFRENCES

[1] Cui, F.; Huo, R.; Hui, G.X.; Lv, J.; Jin, G.; Zhang, X. W. *J. Mol. Stru*. 1001,(**2011**), 104–110.
[2] Tong.C.;. Hu. Z.; Wu. J. *J. Fluore*,20,(**2010**),261-267
[3] Shahabadi, N.; Kashanian, S.; Darabi. F. *Eup. J. Medi. Chem*. 45 (**2010**) 4239-4245
[4] Hartwig. A. *Chemico-Biological Interact.*, 184 (**2010**) 269
[5] Mantle, G. P.;. Marquis, V. F.;. Manderville A. R.; Squillaci,B.;. Pfohl- Leszkowicz .A. *Chem. Res. Toxicol*, 23 (**2010**) 89
[6] Jang .J. K.; Yeo, Y. G.;Cho, S.T.; Eom, G.H.; Kim, C.; Kim, K.S. *Biophys. Chem*. 148 (**2010**) 138
[7] Kashanian,S,;. Askari, S; Ahmadi, F.; Omidfar, K.; Ghobadi, S.; Tarighat A. F. *DNA Cell Biol*. 27(10) (**2008**) 581
[8] Qiao , Y. C.; Bi Y. S.;. Sun, Y.; Song, Q.D.; Zhang, Q.H.; Zhou H.W. *Spectrochim. Acta A.*, 70 (**2008**) 136
[9] Tanious, A. F.; Ding, Y. D.; Patrick, A. D.; Tidwell, R. R.; Wilson, D. W. *Biochem.*, 36 (**1997**) 15315
[10] Ahmadi, F.; Bakhshandeh, F. *DNA Cell Biol*. 28(10) (**2009**) 527
[11] Lipinski, A.C.; Lombardo F.; Dominy, W. B.; Feeney, J. P. *Ad. Drug Delivery Reviews* 46 (**2001**), 3–26
[12] Zhang, Y.; Feng, J.; Chun,xi.; Fang, H.; Wenfan, Xu. *Bio.org & Medi. Chem*. 19 **(2011**), 4437–4444.
[13] Fytas, C.; Zoidis, G.; Tzoutzas, N.; Taylor, C. M. ; Fytas, G.; Kelly, M.J. *J. Med. Chem*. 54 (**2011**), 5250–5254
[14] Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev*. 99(**1999**), 2735–2776
[15] Marks, P. A.; Richon, V. M.; Rifkind, R. A. *J. Natl. Cancer Inst*. 92,(**2000**), 1210–1216.
[16] Zhang G,; Hu, X.; Pan J, *Spectrochimica. Acta Part* A 78 (**2011**), 687-694.
[17] . Bera R,.. Sahoo K B,.. Ghosh S K . Dasgupta S, *Intl. J. Biol. Macromol.* 42 (**2008**)**,** 14-21

[18] M.K. Motlagh, M. Noroozifar and S. Rigi, *Spect. chimica Acta Part* A75 (**2010**) , 598–603
[19] Zhang, G,; Hu, X.; Zhao, N.; Li, W.; Li. He. *Biochem and Phy*, 98, (**2010**),206–21.
[20] Sandstrom, K.;. Warmlander, S.; Leijon, M.; Graslunda, A. *Biochem. Biophys. Res. Commun*., 304, (**2003**). 55-57
[21] Zhong, W.; Yu, H.J.; Liang Y. *Spectrochim. Acta* A. 59 (**2003**) 1281
[22] Pastor, J.; Garcia-Navio G.J.; Vaquero, J.J.; Alvarez-Builla. J.; Gago, F.; Pascual- Teresa,B.; Pastor, M.; Rodrigo, M. M. *J. Org. Chem*.62 (**1997**) 5476.
[23] Girija,C.R.; Karunakar, P.; Poojari, C.S.; Begum, N.S.; Syed, A.A., *J Proteomics Bioinform* 3,**2010**,200-203.
[24] Pande, R.; Tandon, G. S.; *J. Chem. Eng. Data* 24, (**1979**)
[25] Y.T. Wang, F.L. Zhao, K.A. Li, S.Y. Tong, *Anal. Chim. Acta* 396 (**1999**) 75–81.
[26] Ross, P.D.; Subramanian, S. *Biochemistry* 20, (**1981**), 3096–3102.; Nagababu, P.; Satyanarayana S, *Polyhedron* 26 (**2007**) 1686–1692.
[27] Kumar, V.C.; Turner, S.R.; Asuncion, H. E, *J. Photochem. Photobiol. A: Chem*. 74 (**1993**) 231–238
[28] Bi, Y.S.; Zhang, Q.H.; Qiao, Y.C.; Sun, Y.; Liu, M.C. *Spectrochim. Acta*, A 69 (**2008**) 123–129
[29] Sahoo, K.B.; Ghosh, S. K.; Bera, R.; Dasgupta S, *Chem. Phys*. 351 (**2008**) 163–169.
[30] Ghaderi, M.; Bathaie, Z.S.; Saboury, A.A.; Sharghi.; H.; Tangestaninejad, S. *Int. J. Biol. Macromol*. 41 (**2007**) 173–179. http://www.hex6.com