EXPERIMENTAL AND MOLECULAR DOCKING STUDIES ON DNA BINDING INTERACTION OF DERIVATIVE OF N-ARYLHYDROXAMIC ACID

M.A.F.Sumaiya[1], M.I.S.Safeena[2]&R.Senthilnithy[3]

[1]Department of Physical Sciences, Faculty of Applied Sciences, South Eastern University of Sri Lanka, Sri Lanka

[2] Department of Biological Sciences, Faculty of Applied Sciences, South Eastern University of Sri Lanka, Sri Lanka

[3]Department of Chemistry, Faculty of Natural Sciences, Open University of Sri Lanka, Colombo, Sri Lanka

sumaiyamaf@gmail.com safeenim@seu.ac.lk, senthilseusl@gmail.com

ABSTRACT: Binding affinity towards DNA for small molecules such as derivatives of Hydroxamic acids is very important in the development of new therapeuticreagents The binding interaction of N-(o-chloro) phenyl benzoh ydroxamicacid (N-(o-chloro)PBHA) with Ct-DNA was measured by four methods, (i) UV absorption, (ii) fluorescence emission, (iii) viscosity and (iv)molecular docking. It showed that N-(o-chloro) PBHA-DNA complex has less absorption intensity than compound only. Significant enhancement of fluorescence intensity was observed for the N-(o-chloro) PBHA-DNA complex. The values of binding constant, K_b, is $7.87x\ 10^{-3}$ and Stern Volmer constant Ksv is $3.0x10^{-3}$ $ng^{-1}\mu l$ were obtained by UV absorption and fluorescence spectral methods, respectively. The binding interaction trend was further confirmed by viscosity on–Ct-DNA complex which shows the increase in viscosity. The molecular docking of N-(o-chloro) PBHA with the DNA showed the groove binding nature. This observation further confirmed and supplemented the experimental results. The study revealed that the hydroxamic acid derivative seems to have promising anti-cancer drug like nature.

Keywords: Hydroxamic acid, Binding constant, Ct-DNA, Fluorescence, Viscosity, Molecular docking.

1. INTRODUCTION

Significant progress has been made over the past few years in studies of drug-DNA interactions (Rama Pande *et al.*, 2014). The studies on molecular interaction have great importance and become an active research area in recent years. Recently, there has been extreme interest in studies related to the interaction between Drug-DNA, because of their relevance in the development of new reagents for biotechnology and medicine (Manish Pardhi et al., 2016).

Understanding how drugs interact with DNA is of outmost importance as researchers struggle to design more efficient and specifically targeted therapeutics, with fewer side effects (Tamara Topala *et al.*, 2014).

Over the past decade Hydroxamic acids have attracted considerable attention due to their pharmacological, toxicological and pathological properties (Rama Pande *et al*, 2014). They contain a pharmacophoric structural part (-NOH. C=O) (Rita Kakkar, 2013) and Hydrogen Bond Donor (HBD) and Hydrogen Bond Acceptor (HBA), capability to bind with receptors (Agrawal et al., 1999 and Fazary et al., 2001). Derivatives of N-arylhydroxamic acids have also been proved as antitumor/cancer and antioxidant agents.

DNA is the material of inheritance and control the structure and function of cells, which is the major target of most antitumor drugs, as well as many antiviral and antibacterial agents. The possible interaction model between small molecules and DNA generally as follow: (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 (SaherAfshan et al., 2005) and (iii) intercalative binding involves between drug and base pairs (Kashanian *et al.,* 2010).

Many techniques have been applied for investigation of the interaction of DNA with small molecules including UV-Visible Spectrophotometer, fluorescence, CD spectroscopy, X-ray diffraction, Gel electrophoresis and hydrodynamic measurement. The docking is important in the study of various properties associated with protein-ligand interactions such as binding energy and geometry complementarily viscosity (Rama Pande *et al.*, 2012; Deepeshkhare and Rama Pande, 2012).

In this study, both experimental and computational techniques were used to explore the interaction between the N-(o-chloro)phenylbenzohydroxamic acid and DNA.

2. MATERIALS AND METHODS

2.1 Apparatus

The absorption spectra were measured on Genesys 10S UV-Vis spectrophotometer using a 1.0 cm quartz cells. Fluorescence spectra were performed with F-2700 FL spectrophotometer, equipped with a xenon flash lamp using a 1.0 cm quartz cell. Viscosity measurements were carried out using a thermo stated Ostwald viscometer and flow time was measured with a digital stop watch.

2.2 Reagents

The preparation of N-(o-chloro)phenylbenzohydroxamic acid was done by the standard procedure. The purity of synthesized compounds was ascertained by determining their melting point, elementary and IR analysis. Value for M.P, N-(o-chloro)PBHA observed 112^0 C and reported 112^0 C.

Calf thymus DNA (Sigma Aldrich chem, Co. USA) was used without further purification, and its stock solution was prepared by dissolving an appropriate amount of DNA into distilled water. pH of buffer solutions was adjusted with 0.01 M HCl to 7.4 which was prepared by standard procedure. All chemicals were of analytical reagents. The stock solution of 500 ng/µl N-(o-chloro)PBHA was dissolved in DMSO.

2.3 UV Spectroscopic Method

The UV spectra of N-(o-chloro)PBHA and DNA- N-(o-chloro)PBHA complex were measured in the wavelength range of 200- 350 nm. The binding interaction studies were carried out by keeping the fixed concentration of compound (50 ng/µl) to which increasing concentration of DNA stock solution (25 ng/µl- 125 ng/µl) was added. The intrinsic binding constant of the complex N-(o-chloro)PBHA-DNA was calculated from equation,

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f)$$

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] $]/(\varepsilon_b - \varepsilon_f)$ versus [DNA], a slope $1/]/(\varepsilon_a - \varepsilon_f)$ and an intercept $1/ K_b(\varepsilon_b - \varepsilon_f)$.

2.4 Fluorescence Spectroscopic Method

The fluorescence emission spectra were measured in the wavelength range of 320-500 nm upon excitation wavelength at 300 nm using slit with 5 nm each. The fluorescence titrations were performed by keeping the concentration of N-(o-chloro)PBHA constant (50 ng/µl) and varying DNA concentrations (25 ng/µl- 125 ng/µl). A quantitative estimation of fluorescence studies in terms of the Stern-Volmer constant calculation is obtained from Stern Volmer equation,

$$Fo/F = 1+ Ksv [Q]$$

Where, Ksv - Stern Volmer Constant , Q- Concentration of DNA, F_0- Fluorescence intensity in absence of DNA and F -Fluorescence intensity in presence of DNA.

2.5 Viscosity method

The viscosity measurements performed at 25^0 C. A mixture of 15 ml (1.0 ml DNA solution in 14.0 ml Tris-HCl Buffer) was taken in viscometer and flow time read to kept constant DNA concentration (50 ng/µl). An appropriate amount of N-(o-chloro)PBHA added at certain r= [HA]/[DNA] and flow time was measured. The data were presented as $(\eta/\eta_0)^{1/3}$ versus r, where η and η_0 are the viscosity of DNA in the presence and absence of the compounds respectively.

2.6 Molecular Docking

The parameters used for the docking process via HEX 6.3 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

Ligand was drawn and optimized from Gaussian (G09) and converted into PDB file using Avogadro. The DNA duplex receptor structure was obtained from Protein Data Bank (PDB. No: ID1BNA) with 12 base pairs DNA sequence (CpGpCpGpApApTpTpCpGpCpG) running in 3'- 5' directions.

3. RESULTS AND DISCUSSION

Change in absorbance and position of band enabled to observe the possible binding mode of ct-DNA with the compound for calculating the binding constant. Fig. 1 shows the UV absorption of N-(o-chloro)PBHA and DNA-N-(o-chloro)PBHA complex. In the absence of DNA because of π-π* excitation state in N-(o-chloro)PBHA (Manish Pardhi et al., 2016)., it showed a significant absorption peak, situated at 300 nm. When the compound bound to DNA, the intensity was sharply decreasing with hypo-chromic shift. This evidence confirmed binding of N-(o-chloro)PBHA with DNA. The value of Intrinsic binding K_b= 7.87x 10^{-3} was obtained for N-(o-chloro)PBHA.

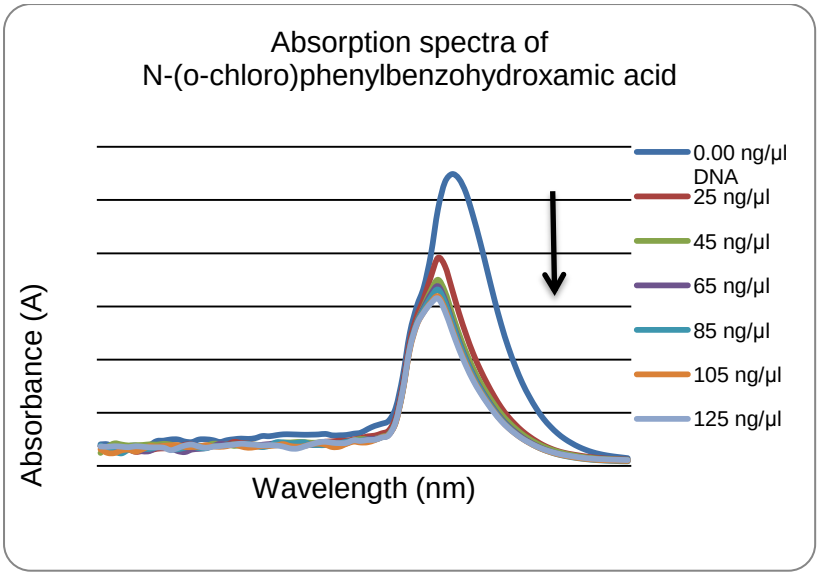

Figure 1. Absorption spectra of N-(o-chloro)PBHA in tris-HCl Buffer in the presence and absence of ct-DNA. N-(o-chloro)PBHA(50 ng/µl); ct-DNA(25-125 ng/µl)

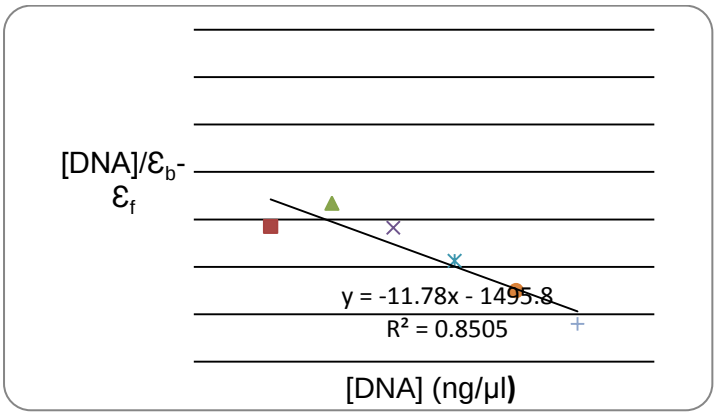

Figure 2. Plot of [DNA]/$\mathcal{E}_b$-$\mathcal{E}_f$ versus [DNA] for N-(o-chloro)phenylbenzohydroxamic)

N-(o-chloro)PBHA exhibits emission spectra in tris- HCl at pH 7.4 buffer solution at ambient temperature with maximum appearing at 341.5 nm (λ_{ex}= 300 nm). The result of fluorescence emission titration for N-(o-chloro)PBHA- DNA were illustrated in Fig 3. The fluorescence of N-(o-chloro)PBHA efficiently enhanced upon binding to DNA and increasing concentration of DNA resulted increase in the fluorescence intensity of N-(o-chloro)PBHA. Thus it was concluded that molecules have been interacted with DNA. In N-(o-chloro)PBHA, presence of chlorine enhances the quenching of emission spectra due to additional intramolecular hydrogen-bond with hydroxyl hydrogen.

The value of Ksvis $3.0x10^{-3}$ ng^{-1}µl for N-(o-chloro)PBHA -DNA complex have been obtained from the plot between Fo/F vs Q as shown in the figure 4.

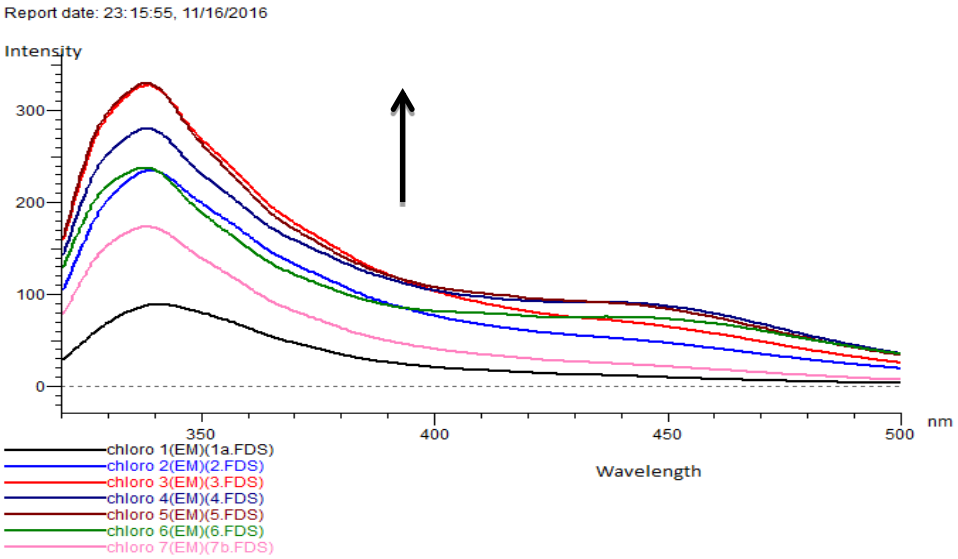

Figure 3. Fluorescence emission spectra of N-(o-chloro)PBHA(50 ng/µl) with increasing amount of DNA(25-125 ng/µl)

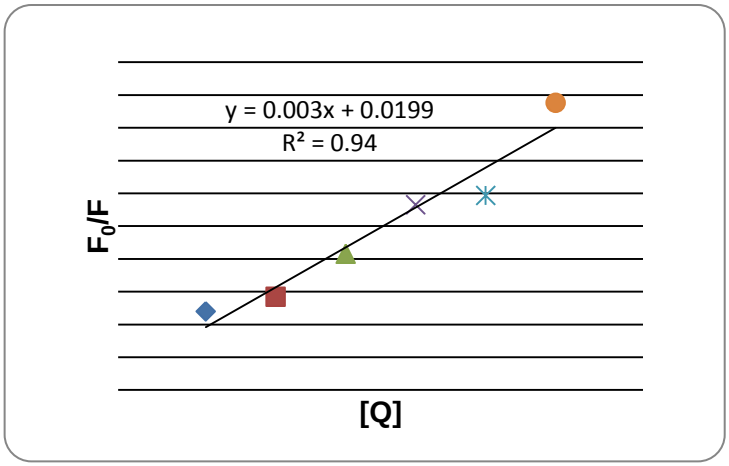

Figure 4. Stern - Volmer plot of N-(o-chloro)PBHA with increasing concentration of DNA

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 apart (Manish Pardhi et al., 2016 and Bharati Verma et al., 2014). It can be seen from the Figure 5, a typical relative DNA viscosity increased with increasing concentration of N-(o-chloro)PBHA. This may be due to the intercalative mode of interaction of compound with DNA.

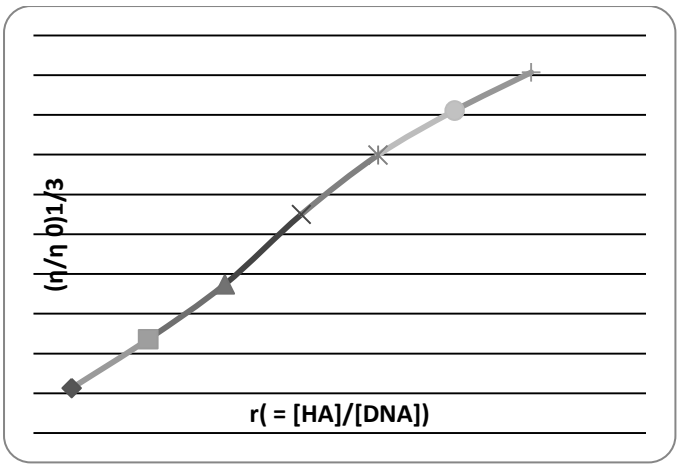

Figure 5. Effect of increasing concentration of N-(o-chloro)PBHA on relative viscosity of DNA.

The structure of the N-(o-chloro)PBHA- DNA complex was predicted from the docking studies using Hex 6.3 software. The structure was studied for various inter-molecular interactions and later subjected to Energy analysis of the complexes. The E_{total} energy obtained -179.33 eV for N-(o-chloro)PBHA- DNA complex (Fig. 7).The Docked Structure act likeN-(o-chloro)PBHAbinds at the minor groove of DNA double helix as shown in Fig 6.

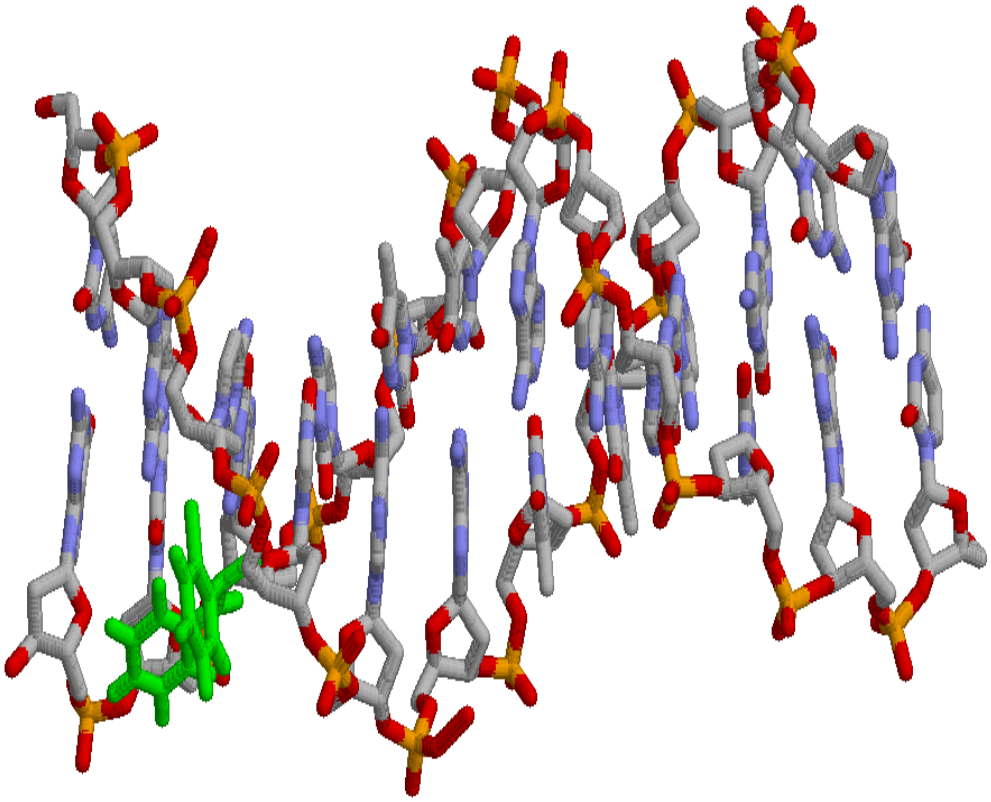

Figure 6. Docked structure of N-(o-chloro)PBHA- DNA complex.

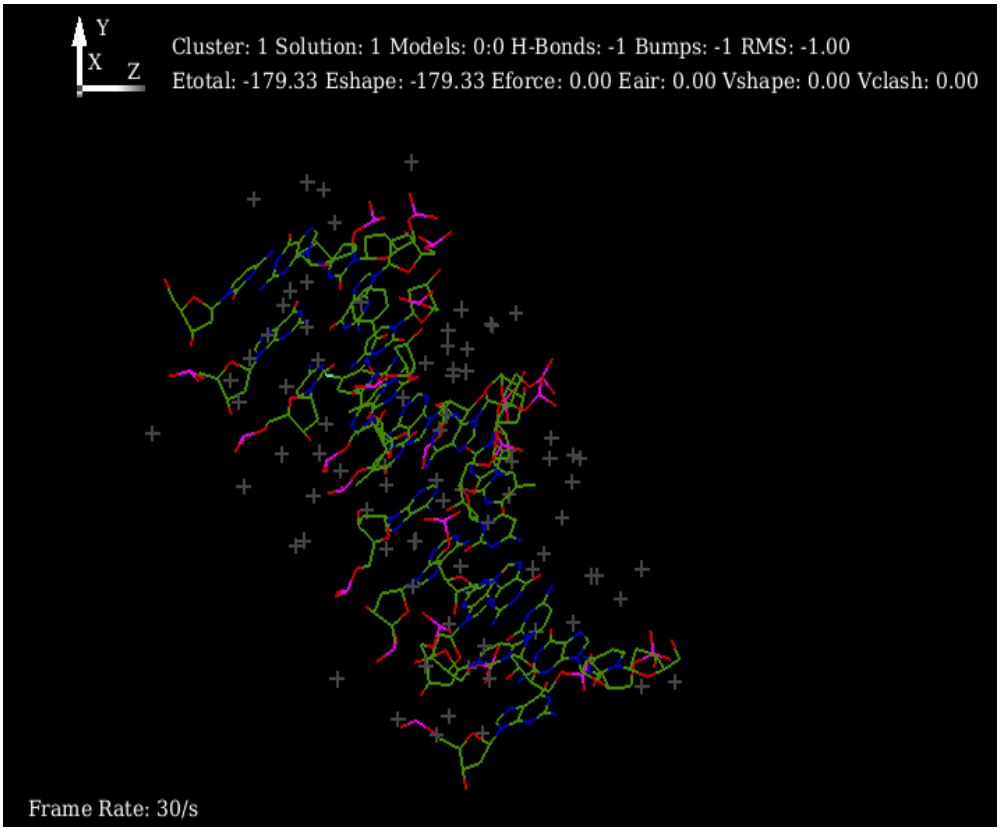

Figure 7. Hex molecular docking shows E_{total} to be -179.33 eV .

4. CONCLUSION

The binding interaction between N-(o-chloro)PBHA and ct-DNA was investigated using UV absorption, fluorescence, viscosity and molecular docking methods. Experimental results indicated that the binding strength of the complexes with ct-DNA exhibited the K_b Value of 7.87x 10^{-3} and Ksv value of 3.0x10^{-3} ng^{-1}µl in in UV absorption and fluorescence emissions respectively. The high binding constants in both experiments showed strong affinity of binding interaction of N-(o-chloro)PBHA with DNA. This may be due to the groove mode of binding while having significant enhancement of fluorescence intensity and linear increase in relative viscosity. Results of molecular docking supplemented and validated the experimental results. This investigation will throw light in the understanding of anti-cancer, anti-tumor and other medicinal properties of derivatives of N-arylhydroxamic acids (N-(o-chloro)PBHA) at the molecular level. The knowledge gained from this study has opened a window for understanding the pharmacological effects of N-(o-chloro)PBHA as a possible and promising drug.

5. REFERENCES

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