A STUDY ON BINDING OF HYDROXAMIC ACID DERIVATIVE TO DNA: EXPERIMENTAL AND COMPUTATIONAL APPROACH

ALF. Jumani[1], MIS.Safeena[2] and 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
alfjumani@gmail.com, safeenim@seu.ac.lk and senthilseusl@gmail.com

Abstract

Small ligand molecules bind to DNA and artificially alter and/or inhibit the functioning of DNA. These small ligand molecules act as drug when alteration or inhibition of DNA function is required to cure or control a disease.The binding interaction of N-phenyllauroylhydroxamic acid with CT-DNA was measured by four methods, (i) UV spectroscopic method, (ii) fluorescence spectroscopic method, (iii) viscosity Measurement and (iv) molecular docking. It showed that N-phenyllauroylhydroxamic acid-DNA complex has high absorption intensity than compound only and significant quenching of fluorescence intensity for the N-phenyllauroylhydroxamic-DNA complex. The values of binding constant, K, is 3.43x $10^{-2}$and Stern Volmer constant Ksv is 4.8x10^{-2} $ng^{-1}\mu l$ obtained by UV absorption and fluorescence spectral methods, respectively. The binding interaction is further confirmed by the increase in relative viscosity of CT-DNA. The molecular docking of N-phenyllauroylhydroxamic acid with the DNA confirmed to be a strong binder with DNA in the mode of groove.

Keywords: Hydroxamic acid, Calf thymus DNA (ct-DNA) and Molecular docking.

Introduction

Small molecules that bind genomic DNA have proven that they can be effective anticancer, antibiotic and antiviral therapeutic agents that affect the well-being of millions of people worldwide.The knowledge of specific targets in rational design of chemotherapeutics is a fundamental factor, principally, for the design of molecules that can be used in the treatment of oncologic diseases. Since the development of cisplatin, DNA became one of the main biological targets for the antitumor compounds (Huang et al.,2005).So the outcome may the cause of DNA damage in cancer cells which results cell death.

Recently small aromatic molecules such as Hydroxamic acid derivatives have become considerable attention in the study of DNA binding interaction with drugs.Hydroxamic acids are a group of naturally occurring or synthetic weak organic acids having the general formula RC(=O)N(R')OH . They are weak proton donors than the structurally related carboxylic acids RC(=O)OH. These acids are widespread in the tissues of plants, metabolites of bacteria and fungi, including complex compounds. Several of the hydroxamic acid derivatives entered into human clinical trials for the treatment of cancer and arthritis (Fazaryet al.,2001).

.

Many small molecules of biological importance are known to interact with DNA involving non-covalent interactions.Three major modes of non-covalent interactions are electrostatic interactions, groove binding and intercalative binding. Electrostatic binding occurs due to interaction of negatively charged DNA phosphate backbone and positively charged end of small molecules. Two different types of groove binding mode are major groove binding and minor groove binding(Mohammed Amir Husain et al., 2015). Groove binding involves hydrogen bonding or van der Waals interaction with nucleic acid bases. Intercalation occurs when small molecules intercalate within the nucleic acid base pairs.

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 dynamic viscosity. 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).

The present study is assessing mode of interaction between hydroxamic acid derivatives and DNA using experimental and computational methods.

Materials and Methods

Apparatus

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

Reagents

The preparation of N-phenyllauroylhydroxamic acid was done by the reported standard procedure. The purity of synthesized compound was ascertained by determining their melting point. Further confirmation will be carried out by FT-IR analysis..Value for melting point(M.P), N-phenyllauroylhydroxamic acid observed 80^0C and reported 80^0C.

Calf thymus DNA (Sigma Aldrich chem., Co. USA) was used without further purification, and its stock solution was prepared by dissolving 1 mg of Ct-DNA in 100 µl Tris buffer and stored at -4^0C. The stock solution (300 ng/µl) of N-phenyllauroylhydroxamic acid was prepared in DMSO. pH of buffer solution were adjusted with 0.01M HCl to 7.4, which was prepared by standard procedure.

UV Spectroscopic method

The UV titrations of the complex were performed using a fixed concentration of the compound N-phenyllauroylhydoxamic acid 50 ng/µl to which increments of the DNA stock solution (25 -110 ng/µl) was added. Absorption spectrum was recorded for compound only (0.00 ng/µl DNA solution).DMSO was used as blank (control). All the resulting solutions were recorded in the wavelength range of 200-350 nm. Binding constant K is used to evaluate the binding strength of N-phenyllauroylhydroxamic acid (Rama Pande et al., 2014)

Fluorescence Spectroscopic Method

Fluorescence emission spectra of N-phenyllauroylhydroxamic acid was recorded at 310 to 500 nm upon excitation wavelength at 290 nm using slit width 5 nm each. The fluorescence titration was performed by keeping concentration of N-phenyllauroylhydroxamicacid constant 50 ng/µl and varying DNA concentration 25 -125 ng/µl.

Emission spectrum was recorded for compound only(0.00 ng/µl DNA solution).DMSO was used as blank (control). Stern volmer constant Ksv is used to evaluate the fluorescence quenching/enhancement efficiency of N-phenyllauroylhydroxamic acid (Rama Pande et al., 2012).

Viscosity Measurement

The viscosity experiments conducted at 298K on thermostated Ostwald viscometer. A mixture of 15 ml (1.0 ml DNA solution in 14.0 ml buffer) was taken in viscometer and flow time noted. An appropriate amount of compound solution was then added into the viscometer to give a certain r = ([HA] / [DNA]) while keeping the DNA concentration constant (Deepeshkhare and Rama Pande, 2012).

Molecular Docking

Hex calculates DNA-N-phenyllauroylhydroxamicacid docking, assuming the ligand is rigid, and it can superpose pairs of molecules using only knowledge of their 3D shapes. 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

The ligand was 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 with sequence d(CpGpCpGpApApTpTpCpGpCpG) running in 3'- 5' directions.

Results and Discussion

UV spectroscopy has been performed to investigate the interaction of N-phenyllauroylhydroxamic acid with ctDNA and investigate the possible binding modes to CT DNA. The absorption spectra were recorded for fixed concentration of N-phenyllauroylhydroxamic acid with increasing concentration of DNA.

In the absence of DNA because of π-π* excitation state in N-phenyllauroylhydroxamic acid , it showed a absorption peak, situated at 290 nm and DNA intensity sharply increase with hyperchromic shift. The hyperchromic effect is the outstanding increase in absorbance of DNA upon denaturation and which has been attributed to the presence of groove surface binding along outside of DNA helix.The result of absorption titration for compound with DNA is illustrated in figure 1.

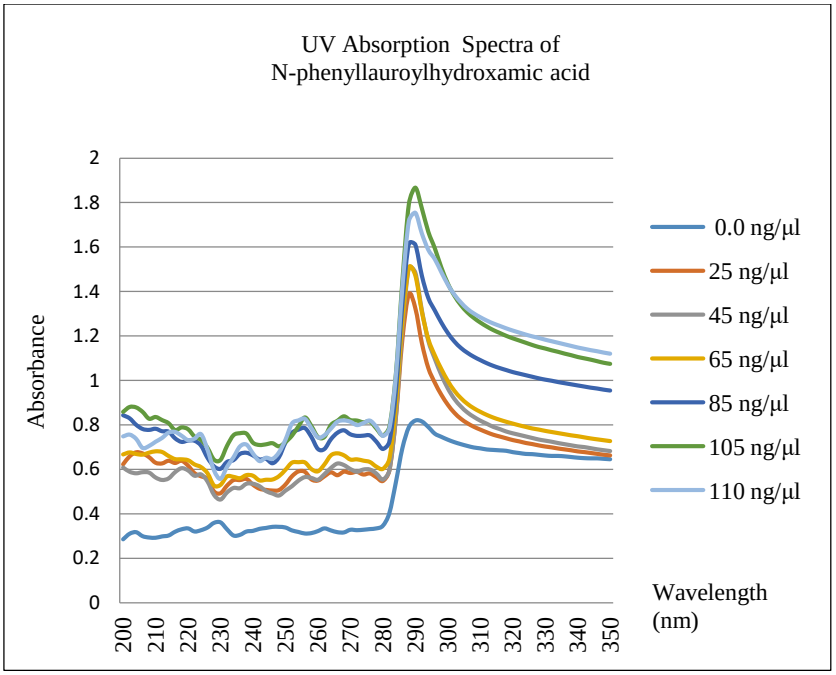

Figure 1: UV absorption spectra of N-phenyllauroylhydroxamic acid in tris-HCl Buffer solution in the absence and presence of DNA

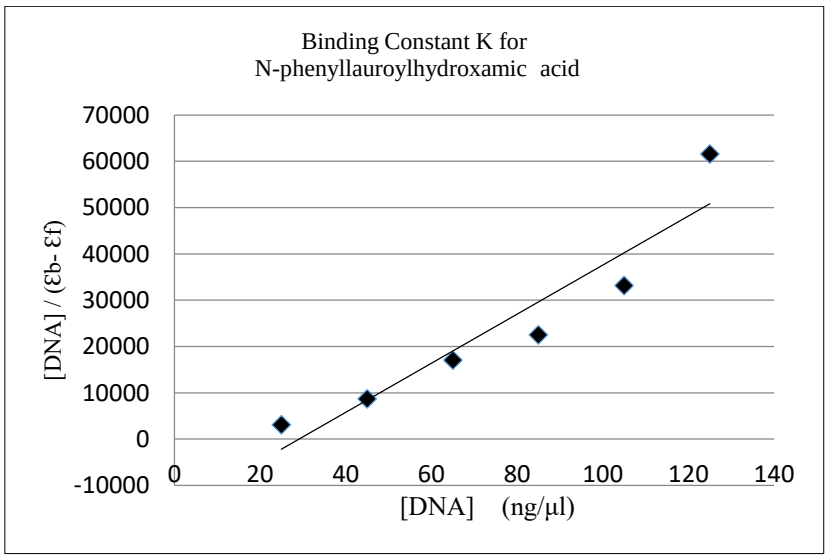

Figure 2: Plot of [DNA]/$Ɛ_b$-$Ɛ_f$ versus [DNA] for N-phenyllauroylhydroxamic acid. The intrinsic binding constant of the complex N-phenyllauroylhydroxamic acid -DNA was calculated from equation.

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

where, [DNA] is base pair concentration, ε_b , ε_f are apparent absorption coefficients for bounded and free DNA .K 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 $1/K(\varepsilon_b - \varepsilon_f)$.The value of Intrinsic binding constant K= 3.43×10^{-2} is obtained for N-phenyllauroylhydroxamicacid (fig.2).

The fluorescence titration spectra have been confirmed to be effective for characterizing the interaction of the compounds with DNA. N-phenyllauroylhydroxamic acid exhibits emission spectra in Tris-HCl with maximum appearing at 339 nm (λ_{ex}= 290 nm). The fluorescence of N-phenyllauroylhydroxamic acid efficiently quenched upon strong binding to DNA and increasing concentration of DNA resulted decrease in fluorescence intensity of N-phenyllauroylhydroxamic acid, which indicated binding interaction with DNA. A result of emission titration for compound with DNA is illustrated in figure 3.

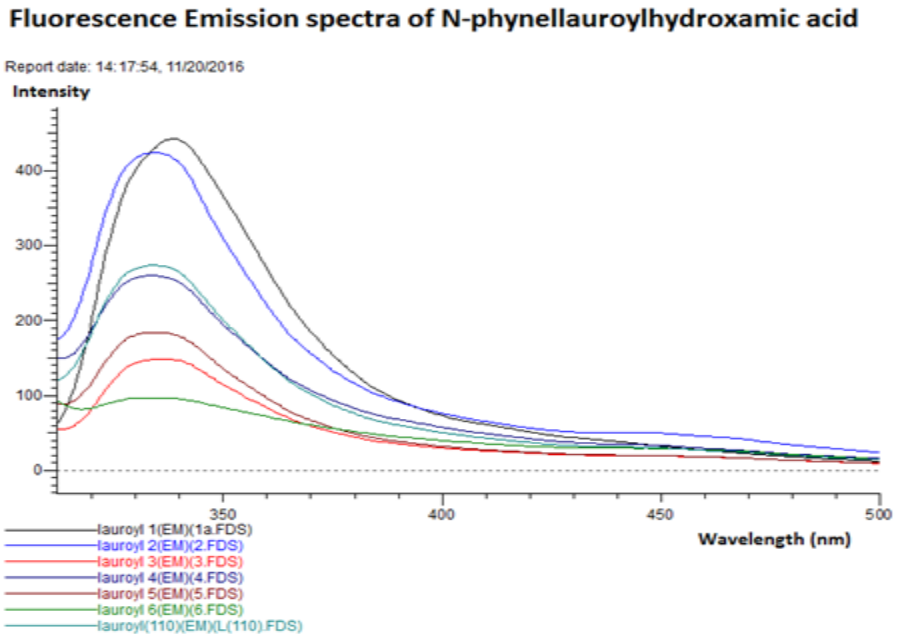

Figure 3: Fluorescence spectra of N-phenyllauroylhydroxamic acid in tris-HCl Buffer solution in the absence and presence of DNA.

To find out the Stern Volmer constant using the equation, $F_0/F = Ksv\,[Q] + 1$ the graph was plotted against (F_0/F) and [Q]. F_0, F –fluorescence intensity at the absence and presence of DNA, [Q]-Concentration of DNA and Ksv-Stern Volmer Constant (Muhammad Sirajuddin, 2013).The value of Ksv is 4.8×10^{-2} $ng^{-1}\mu l$ for N-phenyllauroylhydroxamic acid-DNA complex have been obtained from the plot between Fo/F vs Q as shown in the figure 4.

The interaction between N-phenyllauroylhydroxamic acid and DNA were further confirmed via viscometric measurements. A classical intercalation binding demands the space of adjacent base pairs to be large enough to accommodate the bound ligand and to evaluate the double helix, resulting in an increase of DNA viscosity. As shown in the

figure 5, upon increasing the concentration of N-phenyllauroylhydroxamic acid the relative viscosity of DNA increases. This reflects that the compound has bound to DNA and thereby increasing the viscosity.

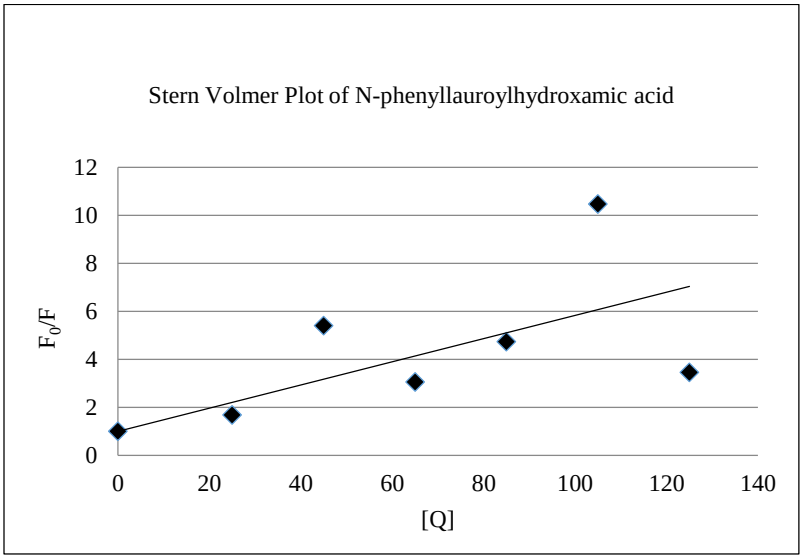

Figure 4. Stern-Volmer plot of N-phenyllauroylhydroxamic acid with increasing concentration of DNA

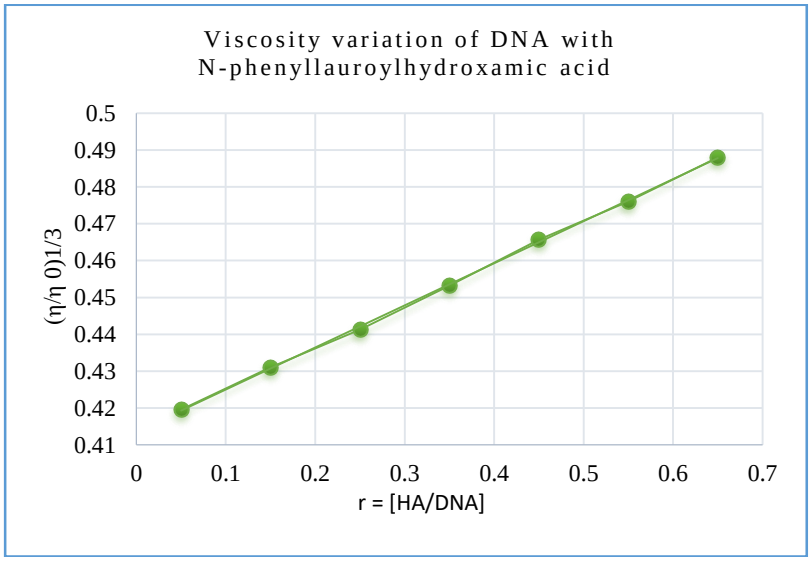

Figure 5: Viscosity variation of DNA at the presence of N-phenyllauroy lhydroxamic acid at increasingamounts (r)

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 and r = ([HA] / [DNA]).

Docking studies of N-phenyllauroylhydroxamic acid with DNA were carried out by Hex 6.3 software. The structure of the N-phenyllauroylhydroxamic acid–DNA complex was predicted. Computational docking showed thatN-phenyllauroylhydroxamic acid act like groove binder which bind to the groove of DNA double helix as shown in figure 6.

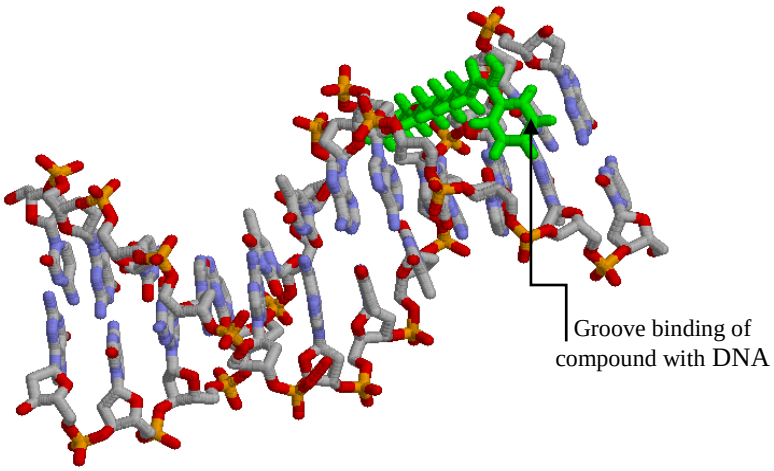

Figure 6. Docked structure of N-phenyllauroylhydroxamic acid - DNA complex.

The E total energy (-237.57eV) was obtained for N-phenyllauroylhydroxamic acid-DNA Complex (fig.7). But the higher E values for N-phenyllauroylhydroxamicacid confirmed that mode of interaction with receptor DNA is intercalator type as per the similar study by Rama Pande et al., 2012.

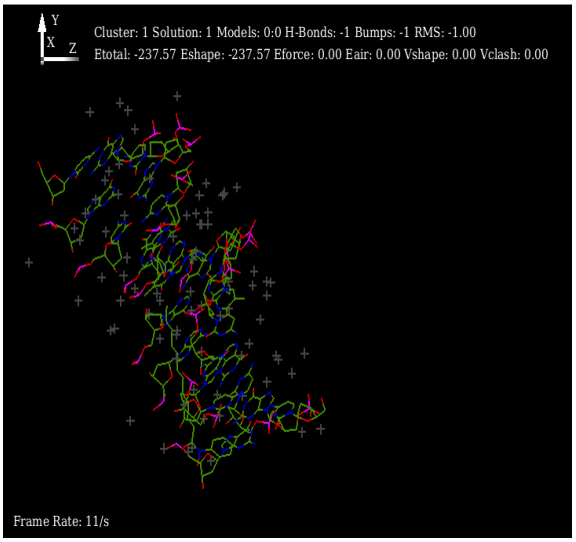

Figure 7. Hex molecular docking shows E_{total} to be -237.57 eV

Conclusion and Recommendation

Experimental results indicate that the binding strength of the N-phenyllauroylhydroxamic acid with Ct DNA calculated with UV and fluorescence spectroscopic titrations have shown the K value 3.43x $10^{-2}$and Ksv4.8x10^{-2} ng^{-1}µl in UV andfluorescence methods respectively. This shows an intercalation mode of binding. But the hyperchromic shift in UV Spectroscopic method and significant quenching in fluorescence spectroscopic method exhibits external groove mode of binding even though the significant increase in relative viscosity of DNA. The computational technique also revealed that compound binds at the minor groove of DNA. So N-phenyllauroylhydroxamic acid shows a strong interaction with DNA.Itmay have a very strong binding affinity with DNA in the groove mode of binding. The knowledge gained from this study will be helpful to understand the interactive mode of hydroxamic acid derivatives in designing of the structure of new and efficient drug molecules.

Acknowledgement

Authors would like to acknowledge the Institute of Chemistry (IChem), Colombo for providing facilities for fluorescence spectroscopy analysis.

References

Deepeshkhare and Rama Pande (2012), "Experimental and molecular docking study on dna binding interaction of N-phenylbenzohydroxamic acid". Der pharma chemia, 4(1), 66-75.

Fazary, AE., Kahlil MM, Fahmy A, &Tantawy TA (2001). The role of Hydroxamic acids in biochemical process, Medical journal of Islamic Academy of sciences 14(3), 107-114.

Huang, R.; Wallqvist, A.; Covell, D. G. (2005). Anticancer metal compounds in NCI's tumor-screening database: putative mode of action. Biochem.Pharmacol, 69, 1009-39. 113.

Muhammad Sirajuddin, Saqib Ali& Amin Badshah.(2013). "Drug–DNA interactions and their study by UV–Visible, fluorescence spectroscopies and cyclic voltametry",Journal of Photochem &Photobiol.124:1-19.

Rama Pande&DeepeshKhare(2012). "Experimental and molecular docking study on DNA bindinginteraction of N-phenylbenzohydroxamic acid", Der Pharma Chemica, 4 (1):66-75.

Rama Pande, BharatiVerma, Manish Pardhi (2014). "Experimental and computational study of binding interaction of Alkoxy Derivatives of N-arylhydroxamic acids with DNA". International Journal of Pharmacy and Pharmaceutical Sciences, pp 171-173.

Sirajuddin,M. ,Saqib Ali and Amin Badshah(2013). "Drug-DNA interaction and their study by UV-Visible, fluorescence spectroscopies and cyclic voltammetry".*journal of photochemistry and photobiology B:Biology*1241-19.