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in vitro Screening of N-Naphthylhydroxamic Acids as DNA Binding Agents

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The *in vitro* interaction between N-1-napthylcaprohydroxamic acid and N-1-napthyllaurohydroxamic acid and ct-DNA was investigated in physiological buffer (7.4 pH) by UV-visible, fluorescence spectroscopy as well as viscosity measurement. The result indicated that both the hydroxamic acid bind to ct-DNA. The binding constant of N-1-naphthylcaprohydroxamic acid and N-1-naphthyllaurohydroxamic acid was found to be respectively 4.1×10^2 L mol^{-1} and 3.4×10^4 L mol^{-1}. The binding constant, number of binding sites and quantum yield were calculated by fluorescence quenching method. Competitive studies with ethidium bromide have shown that the N-1-naphthyllaurohydroxamic acid can displace the ct-DNA–bound ethidium bromide suggesting strong competition with ethidium bromide. The negative value of thermodynamic parameters (ΔG, ΔH, ΔS) and positive value of cell potential (E) showed the spontaneity of the reaction. Binding of hydroxamic acids to ct-DNA was driven mainly by hydrophobic interaction and van der Waals force of attraction. Viscometric studies complimented the spectroscopic studies results, where a small linear increase in the relative viscosity of the ct-DNA solution was observed. The molecular docking was also used to predict the mode of binding of the hydroxamic acids with ct-DNA Conclusively, both hydroxamic acids are found to be strong ct-DNA binders and the modes of binding were intercalative and groove binding for N-1-naphthyllaurohydroxamic acid, N-1-naphthylcaprohydroxamic acid respectively.

Keywords: DNA, Binding constant, Ethidium bromide, Fluorescence, Hydroxamic acid, Buffer.

INTRODUCTION

Uncontrolled rapid multiplication of cells without going to apoptosis phase is the main reason of several deaths. This can only be nursed by inhibiting the frequent procreation of cancer cells, which can be achieved by seizing the replication of DNA. DNA play's a key role in biological systems because it acts as vehicle for hereditary material and control the central machinery of life central dogma, which forms protein. Design of small molecule, which competes with proteins and have ability of attaching to DNA, is main target of researchers. Small molecule DNA complex interaction can trigger the action of DNA and might be possible the interaction may lead to activation or inhibition of target site of DNA which cause or cure disease. Now-a-days, much interest has laid on knowing the mechanism of interaction of small molecules with DNA, so that the outcomes may rule as the cause of DNA damage in cancer cells which results cell death. Investigation drug-DNA interaction play an important role in understanding drug action mechanism.

The fate of interaction between small molecules and DNA probably shows three modes of binding (i) electrostatic binding between cation species and negatively charge phosphate linkage of DNA (ii) groove binding in which molecule binds in groove region of DNA with weak force of binding like hydrogen bonding and van der Waals interaction and (iii) lastly intercalative mode of binding which results due to π-π stacking of drug like molecule within the base pairs [1-7].

Hydroxamic acids possess a wide range of chemical and biological activities. These molecules attract considerable attention because of their outstanding features. These are poly-functional molecules, neutral in nature, consists of pharmacological functionality (-NOH, C=O). Since 1869 after the discovery of hydroxamic acid by Lossen [8] arouse the quest and emphasized on various hidden chemical and biochemical properties of this molecule. In fast few years hydroxamic acids derivatives attain a remarkable focused due to their low toxicity, pharmaceuticals action and reported medicinal property. The histone deacetylase (HDAC) inhibitor suberoyl-anilidehydroxamic acid (SAHA) accelerate human astrocyte neurotoxicity instigated by interferon-γ [9]. Cytotoxicity and IC_{50} value of nineteen N-aryl-substituted hydroxamic acids has been *in vitro* investigated in MCF-7 human breast cancer cell lines by performing MTT assay and obtained range as 61.94 to 337.54 mM. About nineteen hydroxamic acid derivatives were tested in which eighteen molecules were able to inhibit

the growth of cells and values of inhibition was higher than a well-known drug hydroxyurea against same MCF-7 Breast Cancer cell line [10]. The binding interaction of parent molecule N-phenylbenzohydroxamic acid with ct-DNA revealed inter-calations mode of binding [11].

Several techniques like UV-visible spectroscopy, fluorescent spectroscopy, X-ray crystallography, gel electrophoresis, foot printing technique and viscosity are available to determine the drug-DNA interaction. In the present work, we have used UV, fluorescence and viscosity measurement for determining the binding interactions of hydroxamic acids with DNA. The knowledge gained from the work will throw light in the exploration of hydroxamic acids as anticancer as well as antitumor agents.

EXPERIMENTAL

Fluorescence emission spectra were scanned on Cary eclipsed fluorescence spectrophotometer (Varian) using 1 cm quartz cells equipped with xenon flash lamp. Thermo stated Ubbelohole viscometer was used for viscosity measurements. UV spectra measurement performed on Biospectrum BL-198 (Elico) using a 1 cm quartz cells. Temperature controller Gl-635 was used for thermostatically controlled measurement of UV spectrum. All pH measured using Cyber510 digital pH meter with a combined glass-calomel electrode. For centrifugation tarsons spin win micro centrifuge (1.5 mL tube), 6000 rpm was used for the proper mixing.

Hydroxamic acids were synthesized by standard procedure [12]. The purity of synthesized compounds was ascertained by determining their melting points and UV spectra. These data were cross verified from the literature. Particulars of the spectral data and molecular structure are given in Table-1. Hydroxamic acids are sparingly soluble in water, the stock solution (1 mM) of the experimental compounds was prepared in dimethyl sulfoxide for ct-DNA binding studies and stored in a cool and dark place. DMSO from Merck (analytical grade) and calf thymus DNA (Genei, Merck) were used without further purification. It was dissolved in millipore water for the stock preparation and stored at 4 °C. The concentration was determined at 260 nm using UV molar absorption coefficient. The absorbance ratio (A_{260}/A_{280}) was determined for the purification of the DNA [13]. A_{260}/A_{280} was found to be > 1.8, which indicates the protein free nature of DNA. Ethidium bromide was purchased from Merck. 100 mM solution of Tris-HCl buffer was used throughout. 7.4 pH was maintained with 0.01 M HCl prepared by standard protocol. Spectroscopic grade chemicals and millipore water were used for the preparation of solutions.

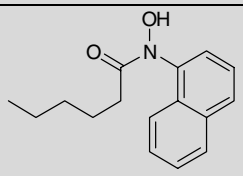
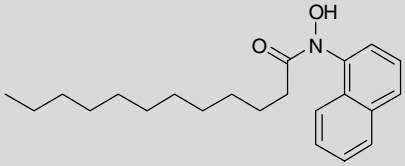
UV spectroscopic titration: The absorption spectra of hydroxamic acids and ct-DNA-hydoxamic acid complexes were measured at various temperatures (298, 303, 305, 308) K in the wavelength range, 200-400 nm. The binding interaction studies were carried out by keeping the amount of hydroxamic acids fixed (100 μM) and titrating with varying concentration of 1 mg/mL ct-DNA from 0 to 125 μL. The reference solution was the corresponding buffer solution. These solutions were allowed to stand for 30 min to equilibrate.

Fluorescence titration: Emission spectra of hydroxamic acids were scanned at 200 to 580 nm upon excitation at 210 nm wavelength using slit width 5 nm each. The concentration of hydroxamic acids solution were kept constant (100 μM) and 1 mg/mL ct-DNA concentration varied from 0 to 125 μL in the titration. For the evaluation of the fluorescence quenching efficiency of hydroxamic acids, Stern-Volmer Constant (K_{sv}) is used.

Ethidium bromide displacement method: The ability of a complex to affect the ethidium bromide (EB) [IUPAC name 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide], fluorescence intensity in the EB-DNA adduct, fluorescence quenching method can be performed to find out the affinity of the complex. Ethidium bromide displacement method was performed by adding 10 μL of 50 μM of ethidium bromide to 0.033 M ct-DNA at λ_{exit} = 475 nm and λ_{em}= 610 nm and fluorescence quenching studies were carried out with varying amount of hydroxamic acids (0-250 μL) that can bind to ct-DNA may modify the binding of ethidium bromide to ct-DNA. The loss of ethidium bromide fluorescence as a function of hydroxamic acid compound has been used for assessing the binding constants of hydroxamic acids and ct-DNA complex.

Viscosity measurement: The viscosity experiments conducted at 298.15 K on thermostated Ubbelohole viscometer, 20 mL of Tris-HCl was transferred to the viscometer and flow time was noted. 1 mL ct-DNA in 19 mL tris-HCl buffer was then taken in viscometer and flow time was recorded. The concentration of ct-DNA was kept constant and an appropriate amount of hydroxamic acid (HA) was added at certain r =

TABLE-1
CHARACTERIZATION OF THE N-1-NAPHTHYLCAPRO AND N-1-NAPHTHYLLAUROHYDROXAMIC ACIDS

Hydroxamic acids	2D Optimized structure	m.f.	m.w. (g)	m.p. (°C)	IR spectra (cm^{-1})	
					ν(O-H)	ν(C=O)
N-1-naphthylcapro-		$C_{16}H_{19}NO_2$	257.33	114	3100	1650
N-l-naphthyllauro-		$C_{22}H_{31}NO_2$	341.49	97	3125	1639

(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 viscosity of ct-DNA in presence and absence of hydroxamic acids.

Molecular docking: Docking is the computational method through which the prediction can be made for the binding of ligand with bio receptors. The molecular docking was done by using HEX Cuda 6.1 software. HEX is the molecular graphics software used for calculating and displaying feasible docking modes of DNA. It necessitates the Ligand and DNA as in front in PDB format. The parameters used for docking include correlation type-shape only, FFT made-3D grid dimension-0.6, receptor range 180, Ligand range 180, twist range-360, distance range-40.

RESULTS AND DISCUSSION

Absorption spectral studies: Measurement of UV-visible absorption is simplest and efficient method in detecting complex formation. In broad, when a small molecule binds with DNA and form a new complex, there are changes in intensity of absorbance and in the position of the band [14]. Fig. 1 shows UV absorption spectra of N-1-naphthylcapro and N-1-naphthyllauro hydroxamic acids in absence and presence of ct-DNA at different r values. Addition of increasing amounts of ct-DNA to derivatives shows hypochromic effect. Similar hypochromism has also been observed for copper(II) complex on interaction with DNA [13]. Generally, the shifts in absorbtion spectra *i.e.* hyperchromism and hypochromism are the spectral features of nucleic acid. With reference of its double helix structure; hyperchromism reveals the breakage of the secondary structure of DNA and hypochromism mean's that the DNA binding mode of molecule is intercalation which can stabilize the DNA duplex structure [15]. Both hydroxamic acids show hypochromic effect due to π-π^* transition on addition of increasing amount of DNA. Hypochromism suggests the close proximity of the compound to the DNA bases [16-18]. Both the hydroxamic acids show a blue-shift, indicating both are binding to ct-DNA. Shifting effect, either in the peak intensity (hyperchromic or hypochromic) or $\lambda_{max\ value}$ (hypsochromic or bathochromic shift) shows interaction between ct-DNA and the hydroxamic acids.

The energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) is increased after the interaction in hypsochromic effect. Hypochromic effect may shows the elongation in the configuration of DNA after the formation of drug-DNA complex. Intercalation mode of small molecules binding to double helix of DNA can revealed by these shifts. Planarity is an important criterion that regulates intercalation of molecules between the base pairs in DNA double helix [19]. Therefore, it is also capable of forming intermolecular H-bonds. All these factors facilitate a stable complex formation between hydroxamic acids and DNA.

Hydroxamic acid–DNA complex, affinity constants (K_a): The intrinsic affinity constant of the hydroxamic acid-ct-DNA complex was calculated from eqn. 1:

$$A_0/(A\text{-}A_0) = \varepsilon_f/(\varepsilon_b - \varepsilon_f) + \varepsilon_f/(\varepsilon_b - \varepsilon_f)*1/K_a[DNA] \quad (1)$$

where, [DNA] is the DNA base pair concentration, A_0 and A are the absorbance of the complex in the free and fully bound

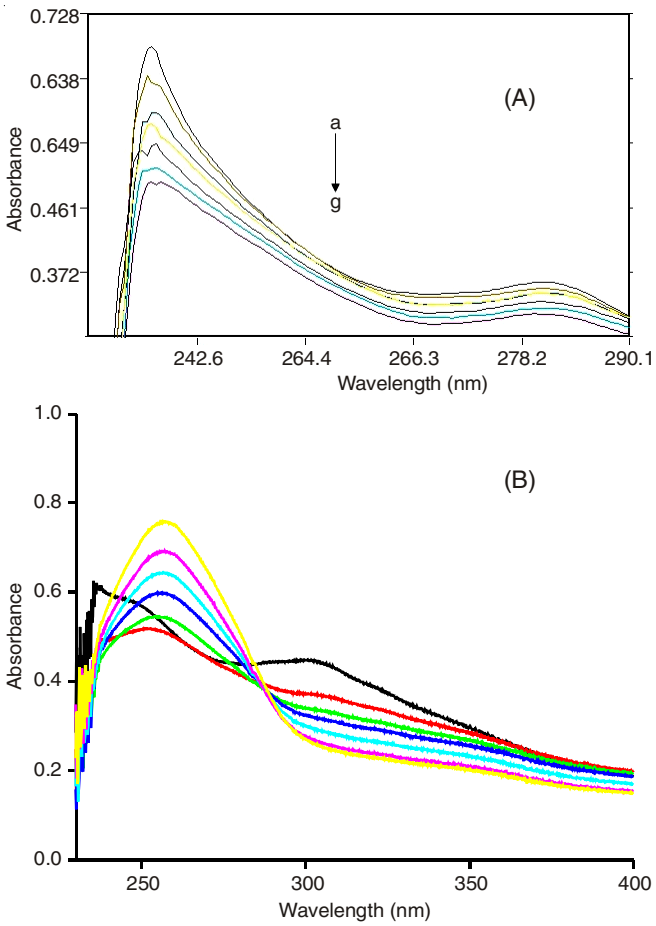


Fig. 1. UV absorption spectra of (A) N-1-naphthylcaprohydroxamic acid and (B) N-1-naphthyllaurohydroxamic acid (100 µM) without and with ct-DNA in DMSO at 298 K. Concentrations of added DNA (µL) were: (a) 0.00 (b) 25 (c) 45 (d) 65 (e) 85 (f) 105 (g) 125

state and ε_f and ε_b are their respective extinction coefficients [20]. K_a was calculated from the ratio of intercept to that of the slope, obtained from a plot of $A_0/(A\text{-}A_0)$ *vs.* 1/[DNA]. The value of intrinsic affinity constant (K_a) for both the hydroxamic acids are 4.1×10^2 M^{-1} and 3.4×10^4 M^{-1} for capro and lauro derivatives of hydroxamic acids. Higher K_a of laurohydroxamic acid indicates that laurohydroxamic acid are strong ct-DNA binder as compared to caprohydroxamic acid.

Thermodynamic parameters: The force of interaction between small molecules and bio-macromolecules mainly include van der Waals force, hydrogen bonds, electrostatic forces and hydrophobic interactions [21]. The evidences for confirming the binding force are thermodynamic parameters of binding reaction. If the enthalpy change (ΔH) does not vary significantly over the temperature range studied, then its value and that of entropy change (ΔS) can be determined from the van't Hoff eqn. 2:

$$\log K = -\Delta H/2.303\ RT + \Delta S/2.303\ R \quad (2)$$

where R is the gas constant. The temperature used were 298, 303, 305, 308 K. The values of enthalpy (ΔH) and (ΔS) were obtained from the slope and the intercept of the linear van't Hoff plot based on log k *versus* 1/T (Fig. 2). The free energy change (ΔG) could be evaluated from the following eqn. 3:

$$\Delta G = -2.303\ RT \log K \quad (3)$$

where *K* is binding constant. The thermodynamic parameters for the interaction of hydroxamic acids with ct-DNA shown in Table-2. The negative *ΔG* values revealed that the interaction process is spontaneous. The values of *ΔH* and *ΔS* obtained (-279870, -930525) and (-983.97, -11005.7) for N-1-naphthylcapro and N-1-naphthyllaurohydroxamic acid, respectively. Hydrogen bond and van der Waals forces are involved in the binding of hydroxamic acids-ct-DNA complex. In accordance with the thermodynamic data, the formation of DNA-hydroxamic acid complex is due to enthalpy favoured condition and there is unfavourable condition for entropy. The complex formation results in a more ordered state due to the size in motion of both DNA and hydroxamic acid [22-24].

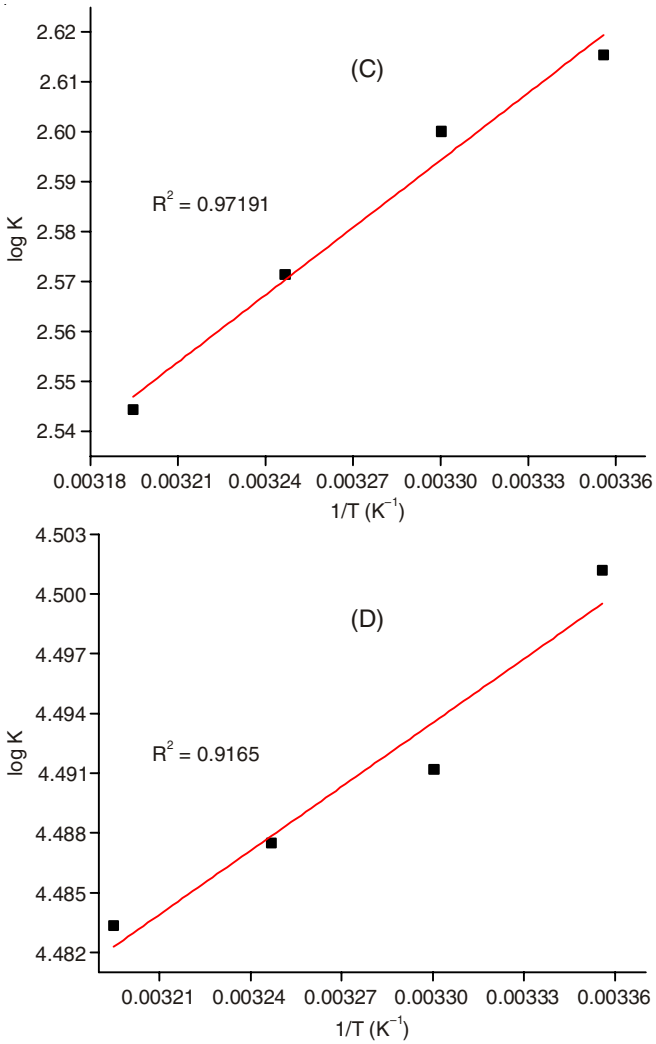


Fig. 2. vant Hoff's plot between log K and 1/T for (C) N-1-naphthylcapro-hydroxamic acid and (D) N-1-naphthyllaurohydroxamic acid respectively

TABLE-2
THERMODYNAMIC PARAMETERS OF N-1-NAPHTHYLCAPRO AND N-1-NAPHTHYLLAUROHYDROXAMIC ACIDS, RESPECTIVELY

Hydroxamic acids	ΔH (kJ mol⁻¹)	ΔG* (kJ mol⁻¹)	ΔS (J mol⁻¹ K⁻¹)
N-1-naphthylcapro-	-279870	-2354020	-983.97
N-1-naphthyllauro-	-930525	-25683.1	-11005.7

*Negative value of Gibb's Free Energy indicates the spontaneity of the binding process.

Viscosity measurements: The DNA interactions of hydroxamic acids were further confirmed *via* viscometric studies. Viscosity measurement is the effective tool for evaluating the binding mode of drugs and DNA. An intercalator requires the space between the adjacent base pairs to accommodate the bound ligand and to lengthen the double helix, resulting in the increase of DNA viscosity [25]. Electrostatic or groove surface binding shows minor effect in the viscosity of DNA [26,27]. The relative viscosity of DNA solution increased linearly (Fig. 3) for both the hydroxamic acids due to lengthening of DNA helix, which may be due to intercalation.

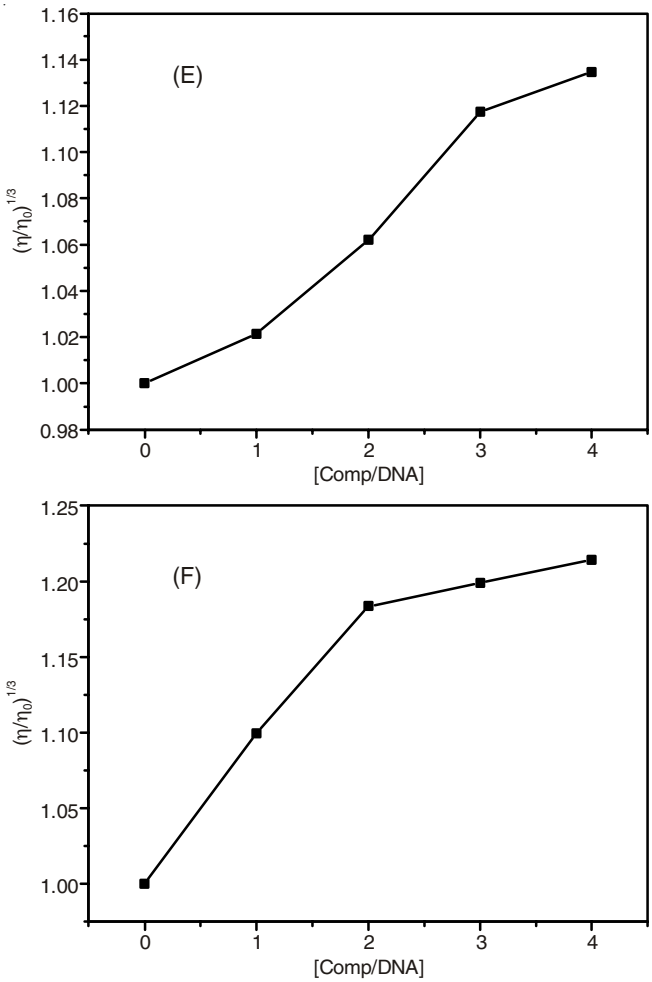


Fig. 3. Effect of increasing concentration of (E) N-1-naphthylcapro-hydroxamic acid and (F) N-1-naphthyllaurohydroxamic acid respectively on relative viscosity with DNA

Fluorescence spectra: Fluorescence spectra of the drugs provide information about their localization and interaction mode with DNA. Fluorescence emission spectra significantly enhanced by increasing the ct-DNA concentration [28]. Both of the compounds gave a fluorescence emission peak at 423.93 nm in Tris-HCl buffer solution at pH 7.4 at room temperature. The results show that the fluorescence spectra of laurohydroxamic acid could be quenched by ct-DNA resulting in gradual decrease in emission intensity of hydroxamic acids (Fig. 4) whereas on interaction of caprohydroxamic acid ct-with DNA caused a significant concentration-dependent increase of the fluorescence intensity. Merely binding to phosphate backbone or to the grooves of DNA cannot result in a significant enhance-

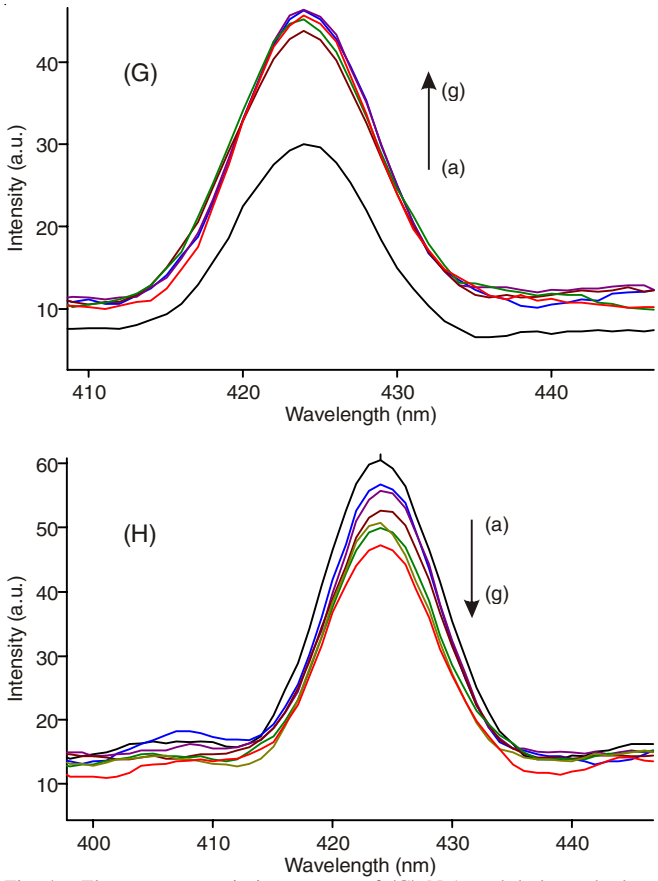


Fig. 4. Fluorescence emission spectra of (G) N-1-naphthylcaprohydroxamic acid and (H) N-1-naphthyllaurohydroxamic acid with DNA. A compound concentration (100 μM) in Tris-buffer, pH 7.4. DNA concentration (μL) (a) 0.00 (b) 25 (c) 45 (d) 65 (e) 85 (f) 105 (g) 125

ment of the fluorescence polarization. Thus, it is concluded that the molecules have been interacted with the ct-DNA where they are protected from aqueous environment due to its hydrophobic nature. This causes lesser mobility of the molecule further leading to lesser vibrational relaxation during the excited state [29-31]. This association involves the insertion of a planar fused aromatic ring system between the DNA base pairs, leading to significant π-electron overlap. Moucheron and Mesmaeker [32] reported that this mode of binding is usually favoured by the presence of an extended fused aromatic ligand.

Binding constant (K_b) and number of binding sites (n): The binding stoichiometry of the DNA-hydroxamic acids complex was calculated from following eqn. 4. It signifies the number of bound DNA per hydroxamic acid molecule.

$$\log (F_0\text{-}F)/F = \log K_b + n \log [DNA] \quad (4)$$

The value of K_b was obtained from the graph plotted between log $[(F_0\text{-}F)/F]$ *versus* log [DNA] (Fig. 5). F_0 is the fluorescence intensity of compound alone, while F is the fluorescence intensity of hydroxamic acid with the presence of DNA. K_b and n of complex at 298 K has been calculated are shown in Table-3. The geometry of the drug binding to DNA can be characterized by the binding stoichiometry of the hydroxamic acid-DNA complexes [33]. Higher number of binding sites and K_b for laurohydroxamic acid suggests that the mode of binding is intercalation.

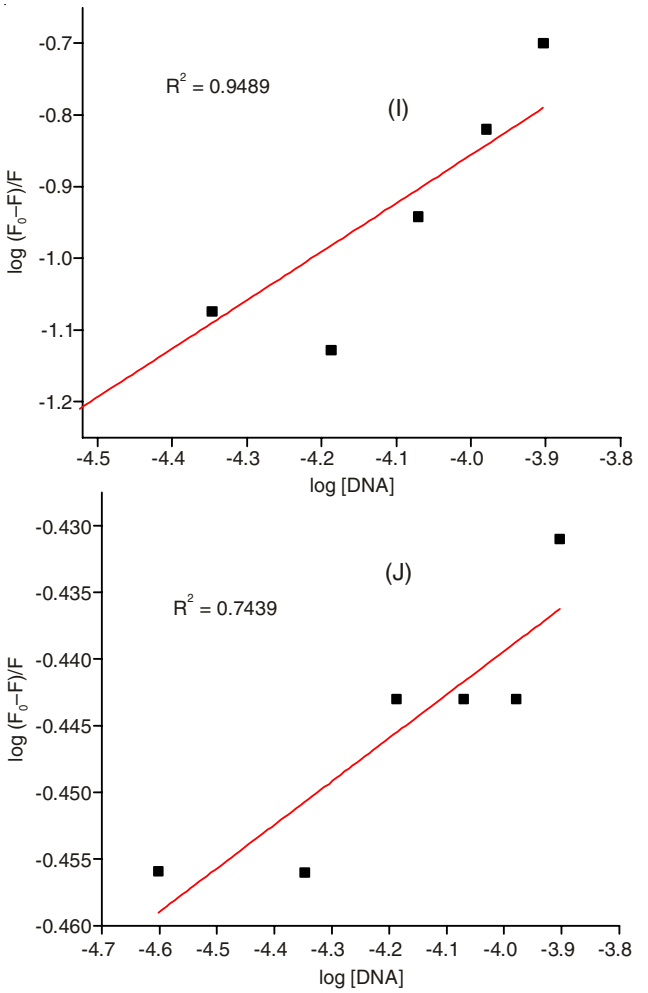


Fig. 5. Graph plotted between log $(F_0\text{-}F)/F$ *versus* log [DNA] for (I) N-1-naphthylcaprohydroxamic acid and (J) N-1-naphthyllaurohydroxamic acid

TABLE-3
BINDING CONSTANT AND NUMBER OF BINDING SITES N-1-NAPHTHYLCAPROHYDROXAMIC ACID AND N-1-NAPHTHYLLAUROHYDROXAMIC ACID

Hydroxamic acids	Binding constant K_b (M^{-1})	Number of binding sites (n)
N-1-naphthylcapro-	0.489	0.032
N-1-naphthyllauro-	1096.45	0.9264

Ethidium bromide displacement method: The displacement of ethidium bromide bound to ct-DNA by the drug molecules has been used to analyze ct-DNA binding ability as it shows intercalative mode of binding with ct-DNA due to its planar structure [34]. ct-DNA has native fluorescence, but under the usual working conditions this is negligible and hence the fluorescence intensity depends on the amount of a fluorescent dye like ethidium bromide. The presence of other compounds that can bind to DNA may modify the binding of ethidium bromide to DNA. The loss of ethidium bromide fluorescence as a function of added compound has been used for assessing the binding mode of hydroxamic acids with ct-DNA. The emission maxima were obtained at 597.94 nm. The emission spectra of EB-DNA system in the presence and absence of N-1-naphthylcaprohydroxamic acid and N-1-naphthyllaurohydroxamic acid are shown in Fig. 6.

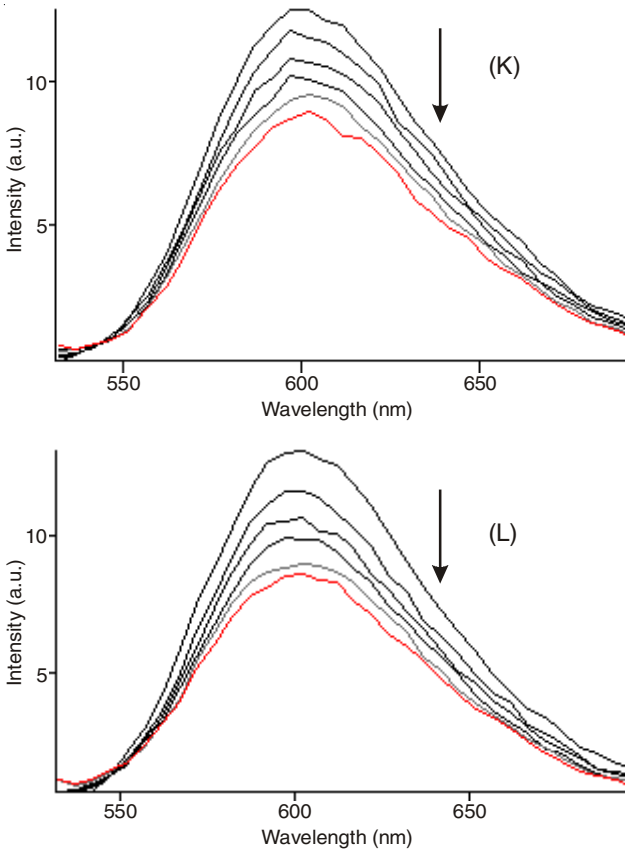


Fig. 6. Emission spectra of ethidium bromide bound to DNA in presence of (K) N-1-naphthylcaprohydroxamic acid and (L) N-1-naphthyl-laurohydroxamic acid in Tris-HCl buffer (pH 7.4)

The Stern-Volmer quenching plots from the fluorescence titration data of ethidium bromide bound to DNA [35-37] and the hydroxamic acids were investigated according to the Stern-Volmer eqn. 5:

$$F_0/F = 1 + K_{sv}[Q] \quad (5)$$

where F_0 and F are the fluorescence intensity of ethidium bromide bound to DNA in the absence and presence of the quencher. [Q] is the ratio of the concentration of hydroxamic acid with DNA. The Stern-Volmer quenching constant (K_{sv}), for ethidium bromide bound to DNA has been determined from the plot of F_0/F *versus* [Q]. The quenching plots of F_0/F *vs.* [Q] (Fig. 7) are in good agreement with the linear Stern-Volmer equation with K_{sv} values of 0.03972 and 0.0537 for N-1-naphthylcapro and N-1-naphthyllaurohydroxamic acids respectively. Higher K_{sv} for laurohydroxamic acid as compared to caprohydroxamic acid indicates that laurohydroxamic acid is able to displace ethidium bromide from EB-DNA complex. Hence, laurohydroxamic acid is an intercalator.

Quantum yield (Q): It stands as the amount of energy transferred from DNA to compound upon binding. Its value was estimated from the ratio of the quantum efficiency of hydroxamic acid bound to ct-DNA (q_b) and that of the free hydroxamic acid (q_f) using the following eqn. 6:

$$Q = \frac{q_b}{q_f} = \frac{I_b}{I_f} \times \frac{\varepsilon_f}{\varepsilon_b} \quad (6)$$

where I_b and I_f stands for the fluorescence intensities of bound and free ligand and ε_b and ε_f stands for the molar extinction

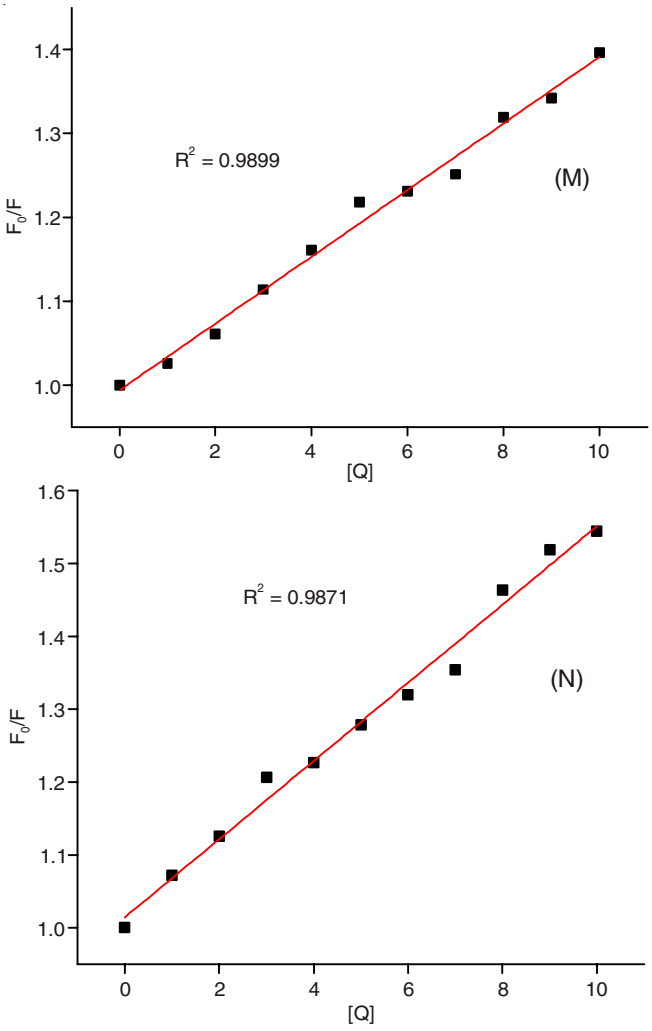


Fig. 7. Quenching plots of F_0/F *vs.* [Q] for (M) N-1-naphthylcaprohydroxamic acid and (N) N-1-naphthyllaurohydroxamic acid

coefficients for the bound and free ligand [38,39]. The values of quantum yield of both the hydroxamic acids are tabulated in Table-4. This denotes that both the molecules exhibit greater fluorescence in the bound state as compared to the Free State in solution, *i.e.*, the loss of fluorescence energy is difficult when bound to the DNA helix.

TABLE-4
SHOWS THE QUANTUM YIELD OF N-1-NAPHTHYLCAPRO AND N-1-NAPHTHYLLAUROHYDROXAMIC ACIDS

Hydroxamic acid	$*\varepsilon_f$ (μM^{-1} cm^{-1})	$**\varepsilon_b$ (μM^{-1} cm^{-1})	Quantum yield
N-1-naphthylcapro-	0.02	0.01	2.40
N-1-naphthyllauro-	0.02	0.02	0.93

$*\varepsilon_f$ and $**\varepsilon_b$ = molar extinction coefficients for the bound and free hydroxamic acid respectively

Standard cell potential of hydroxamic acid-DNA interaction: To study the interaction mechanism, the pre/post electrochemical signals of nucleic acid or small molecule interaction can be observed. The technique exploits the sensitivity of a long-range charge transfer observed through double stranded DNA, utilizing "on" and "off" switching of such a charge transfer upon drug binding as the basis to probe the

electronic changes that occur to the DNA upon drug exposure. Disruption of the DNA base-pair stack will reduce the efficiency of the charge transfer to the redox species. A redox couple is build between the DNA nucleobase and the hydroxamic acid as a result of which a cell potential is setup between the DNA and hydroxamic acid. Cell potential was calculated by the eqn. 7:

$$\Delta G = -nFE \quad (7)$$

where, ΔG is the standard free energy change of the reaction and n is the number of electrons exchanged in the reaction (for hydroxamic acids its value is 1), F is the Faradays constant, E is the standard cell potential of the couple. The value of standard cell potential for N-1-naphthylcaprohydroxamic acid and N-1-naphthyllaurohydroxamic acid are 0.155 and 0.275 volts, respectively. This relationship serves as a bridge between thermodynamics and electrochemistry. The positive value cell potential suggested that the reaction is spontaneous.

Molecular docking: Docking studies of N-1-naphthylcaprohydroxamic acid and N-1-naphthyllaurohydroxamic acid with dsDNA were carried out. The structure of the compound–DNA complex was predicted. In the present work docking studies predicts that N-1-naphthyllaurohydroxamic acid having, planar ring structure intercalates between nitrogenous base pairs of DNA while N-1-naphthylcaprohydroxamic acid acts like groove binder which binds to the groove of DNA double helix as shown in Fig. 8. The E_{total} energy obtained is -261.64 eV and -360.66 eV for N-1-naphthylcaprohydroxamic acid and N-1-naphthyllaurohydroxamic acid respectively.

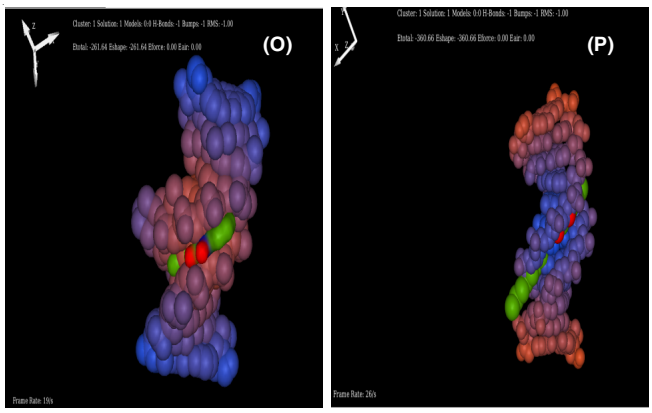


Fig. 8. HEX molecular docking of (O) N-1-naphthylcaprohydroxamic acid and (P) N-1- naphthyllaurohydroxamic acid

Conclusions

In the present work we have explored the binding interaction of N-1-napthylcaprohydroxamic acid and N-1-napthyllauro hydroxamic acid with DNA in the physiological buffer by UV-visible and fluorescence spectroscopic techniques as well as by viscometric method. It has been examined that the napthyl derivative of hydroxamic acid on the elongation of alkyl derivative chain has the drastic effect on the mode of binding. The N-1-napthyllaurohydroxamic acid has higher binding constant having long chain than N-1-napthylcaprohydroxamic acid with shorter chain. Hydroxamic acids showed one band in its UV spectrum signifying π-π^* transition. On addition of various concentrations of DNA, hypsochromic shift in all UV spectra were observed. It was found that the binding constant decreases significantly on increasing the temperature. The results of the fluorescence titration revealed that DNA had strong ability for quenching the intrinsic fluorescence for N-1-napthyllauro hydroxamic acid in comparison to N-1-napthylcaprohydroxamic acid. Ethidium bromide competition fluorescence assay of both the hydroxamic acid's illustrated that the complex could strongly bind to DNA as an intercalator competing with ethidium bromide. The calculated thermodynamic parameters revealed that the binding of hydroxamic acids to DNA was driven mainly by hydrogen bonds and van der Waals forces. The negative values of *ΔG, ΔH, ΔS* suggest the spontaneity of the reaction. The positive value of cell potential E further confirmed the same effect.

Viscometric studies revealed that there is increase in the relative viscosity of both the compounds indicating the lengthening of DNA helix and the mode of binding. Thus out of two hydroxamic acids, N-1 napthyllauro hydroxamic has more intercalation than N-1-napthylcaprohydroxamic acid on interaction with DNA. Molecular docking complemented on the spectroscopic results. This knowledge is helpful in the screening of the various hydroxamic acids as anticancer agent and for other pharmacological effects. This will also help to build up a more effective and the potent drug for the nursing of cancer.

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