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# Ternary Cu(II) – Salicylidene Amino Acid Schiff base – Heterocyclic base Complexes as DNA Binding Agent : a Comparative Study

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**Abstract:** Deoxyribonucleic acid (DNA) is a molecule carrying genetic instructions for the development, functioning, growth and reproduction of all known organisms and many viruses. Hydrolysis of phosphodiester bond of DNA (DNA cleavage) is of critical importance at several stages in cell cycle and design of artificial metallonucleases.DNA binding is important for nuclease to exhibit DNA cleavage activity. Hence an attempt is made in this article to compare the DNA binding abilities of few amino acid based schiff base complexes reported earlier. Ternary Cu(II) - schiff base - heterocyclic base complexes [Cu(II)(saltrp)(phen)] (**1**), [Cu(II)(saltrp)(bipy)] (**2**), [Cu(II)(saltyr)(phen)] (**3**) and [Cu(II)(saltyr)(bipy)] (**4**) (where saltrp/saltyr = Schiff base derived from salicylaldehyde (sal) and amino acids – tryptophan (trp) or tyrosine (tyr); phen = 1,10 phenanthroline and bipy= 2,2' bipyridine) were synthesized and characterized. Complexes (**1-4**) exhibited square pyramidal geometry ($CuN_3O_2$) where Schiff base (saltrp and saltyr) and heterocyclic base act as ONO and as NN donor ligands respectively. Calf thymus (CT) DNA ability of complexes (**1-4**) studied by various techniques revealed that the complexes bind through intercalative mode and show good binding ability. The DNA binding ability of the complexes follow the order **1**>**2**>**3**>**4**. Phenanthroline complexes (**1,3**) binds DNA more effectively as compared to their corresponding bipyridine (**2,4**) complexes due to the presence of extended aromatic phenyl ring which might be involved in an additional stacking interaction with DNA bases.

**Keywords:** Schiff base, Copper complexes, DNA binding, Absorption & Fluorescence Spectroscopy.

## I. INTRODUCTION

Deoxyribonucleic acid is the site of storage and retrieval of genetic information through interaction with proteins and other small molecules. DNA is the primary target for many anticancer drugs in tumor cells [1,2]. Hence development of artificial metallonucleases that bind and cleave DNA has gained much attention. Metal based compound cisplatin is one of the most widely used drugs for the treatment of cancer [3], but it is associated with the high toxicity leading to severe side effects and acquired resistance [4]. Owing to the limitations in the usage of cisplatin, development of anticancer drugs of other metals [5,6] have gained much importance which can be promising alternatives to platinum in cancer therapy and are less toxic and more effective for chemotherapeutic application. Many transition metal complexes are known to bind to DNA via both covalent and non-covalent interactions and cleave DNA mimicking the function of endonucleases [7-10]. In covalent binding the labile ligand of the complexes is replaced by a nitrogen base of DNA. On the other hand, the non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of cationic metal complexes outside of the DNA helix, major or minor groove.

Copper, a natural constituent of cell nuclei has been suggested to play a key role in structural organization [11] and function [12] of chromosomes. Several copper complexes with wide range of ligand environments has been proven to be effective in DNA binding and cleavage [13-16]. Schiff bases, an important class of ligands in coordination chemistry are able to inhibit the growth of several animal tumors, and some metals complexes with schiff bases have shown good antitumor activity against animal tumors [17,18] thereby showing extensive applications in pharmacology [19]. Several metal complexes of salicylidene amino acid schiff base [20-22] and reduced salicylidene amino acid [23,24] were reported and few of them have been proven to be efficient DNA cleavage agents [25,26] and as tumor chemotherapeutic and tumor radio imaging agents [27].

Considering the fact that schiff base complexes have significant importance in the design of artificial nucleases, we have made an attempt in this article to analyze and compare the DNA binding abilities of a few schiff base complexes reported from our laboratory. Complexes studied in the present series have an ensemble of biologically significant metal ion, copper; a tridentate salicylidene amino acid schiff base ligand (saltrp, saltyr); polypyridyl ligands (phen, bipy) with varying intercalating ability.

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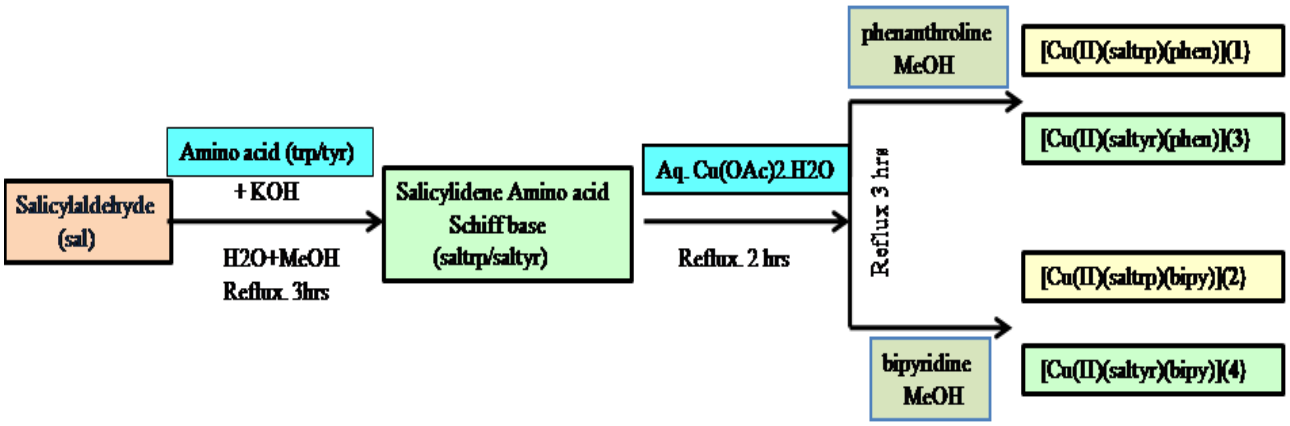
## II. MATERIALS

**General:** Chemicals and solvents (Spectroscopic grade) required for the synthesis of ligands and complexes were purchased from Sigma and other commercial sources. Calf Thymus (CT) DNA for binding studies was obtained from Fluka (Switzerland).

## III. EXPERIMENTAL

### *A. Synthesis and characterization of metal complexes*

Ligands and complexes (**1-4**) reported from our laboratory [28, 29] were synthesized by adopting a method published earlier with slight modifications [25] **(scheme 1).**




**Scheme 1. Synthesis of complexes 1-4**

Complexes (**1-4**) were characterized by elemental analyses, Conductivity measurements, Magnetic moment and spectroscopic techniques like ESI-MS, IR and UV-Vis spectroscopy **(Table 1).** The analytical data for the complexes were in good agreement with the proposed molecular formulae.

**Table 1. Physicochemical Data for Complexes 1-4**

| Complex | IR ($cm^{-1}$)[a] $\upsilon$(C=N) | $\mu_{eff}$[b] (BM) | ESI-MS (m/z) | UV-vis[c] ($\lambda_{max}$, nm) | $\Lambda_M$[d] ($\Omega^{-1}Cm^2\ M^{-1}$) |
|---|---|---|---|---|---|
| [Cu(II)(saltrp)(phen)](**1**) | 1627 | 1.82 | 550 | 222,266,356,664 | 12 |
| [Cu(II)(saltrp)(bipy)](**2**) | 1625 | 1.79 | 526 | 218,273,354,667 | 13 |
| [Cu(II)(saltyr)(phen)](**3**) | 1633 | 1.78 | 527 | 223,268,394,665 | 10 |
| [Cu(II)(saltyr)(bipy)](**4**) | 1636 | 1.80 | 503 | 220,274,388,660 | 12 |

[a] IR (KBR phase); [b] Magnetic moment; [c] UV-Vis spectra in MeOH; [d] Molar Conductance in MeOH

Based on the characterization data, structures were proposed for complexes **1-4**. The coordination geometry around Cu(II) was distorted square pyramidal ($CuN_3O_2$) with the schiff base (saltrp/saltyr) coordinating to the Cu(II) through phenolate oxygen (O), azomethine nitrogen (N) and carboxylate oxygen (O). The neutral phen/bipy ligands coordinated to Cu(II) through N, N donor atoms **(Figure 1a).** Green single crystals of [Cu(II)(saltrp)(bipy)] (**2**) were grown by slow evaporation of methanolic solution of **2** for a week and was structurally characterized by single crystal X-ray crystallography [28] **(Figure 1b).**

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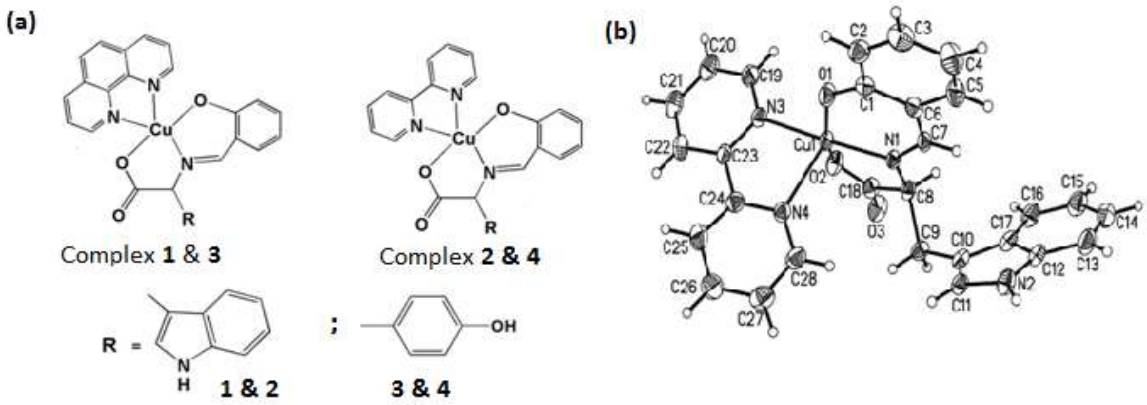


**Figure 1. (a) Molecular Structures of complexes 1-4.**

**(b) Crystal Structure of complex 2**

***B. DNA Binding Study***

Interaction of complexes (**1**-**4**) with CT-DNA was studied by various techniques viz. absorption spectroscopy, Thermal denaturation, fluorescence spectroscopy and viscosity. The DNA binding mode and affinity was established based on the results obtained [28,29].

## IV. RESULTS, DISCUSSION AND COMPARISION

The DNA binding of complexes [Cu(II)(saltrp)(phen)] (**1**), [Cu(II)(saltrp)(bipy)] (**2**), [Cu(II)(saltyr)(phen)] (**3**), [Cu(II)(saltyr)(bipy)] (**4**) have been reported from our laboratory [28,29]. Since DNA binding by complex is a prerequisite for its DNA cleavage activity, a brief summary of DNA binding techniques adopted along with the trends observed for **1**-**4** is made in this article.

***A. DNA binding by absorption spectral studies:***

Electronic absorption spectroscopy is one of the most common ways to investigate the interaction of complexes with DNA. The UV absorption spectra of the complexes in the absence and presence of CT DNA were monitored. In the presence of increasing amounts of CT-DNA (0-10μM), a decrease in the intensities of absorption (Hypochromism) with absorption intensity shift towards longer wavelength (bathochromism) of ~2 nm was observed for **1**-**4** (**Figure 2** (**1** & **3**). Hypochromism and bathochromism are indicative of intercalative mode of binding where the aromatic chromophore of the complex stacks between base pairs of DNA [30-33].

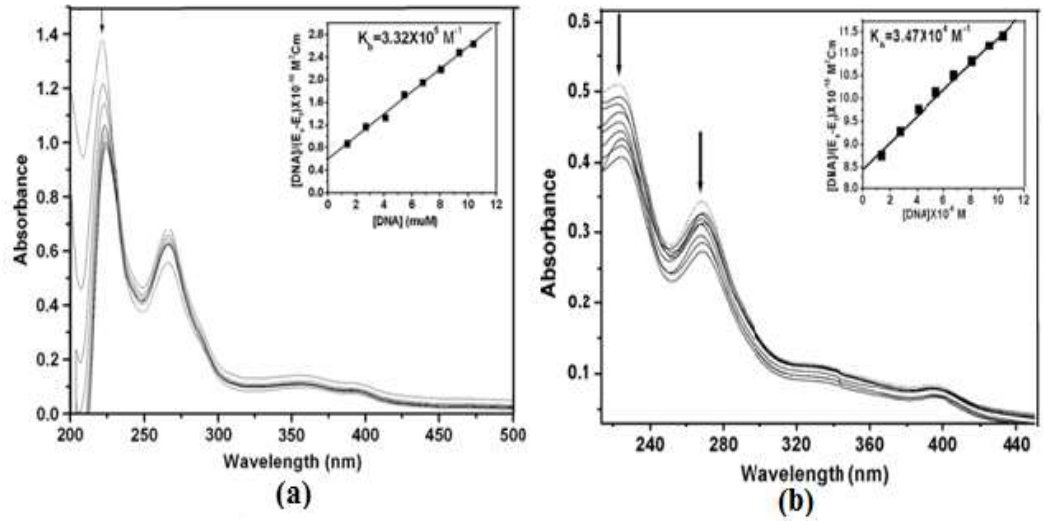


**Figure 2.** Absorption spectra of 1(a) & 3(b) in the absence (·····) and presence (—) of increasing amounts of CT-DNA. Conditions: [Cu] = 10 μM, [DNA] = 0–10 μM. Arrow (↓) shows the absorbance changes upon increasing DNA concentration. Inset: linear plot for the calculation of the intrinsic DNA binding constant, $K_b$ **.**

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Intrinsic binding constants ($K_b$, insets of resp. Figure 2) for the complexes with CT-DNA were determined using the following functional equation [34,35]

$[DNA]/(e_a-e_f) = [DNA]/(e_b-e_f) + 1/ K_b(e_b-e_f)$ -------(1)

The observed $K_b$ values (**Table 2**) for **1-4** were comparable to that observed for classical intercalator, Ethidium bromide (EB-DNA, $1.4\times10^6\ M^{-1}$) [36].

**Table 2. DNA Binding Data for Complexes 1-4.**

| Complex | UV-Vis absorption | | Thermal Denaturation | | Fluor. Quen. |
|---|---|---|---|---|---|
| | [a] Change in Abs. | [b]%H | [c]$K_b$ | [d]$\Delta T_m$ | [e]$K_{app}$ |
| [Cu(saltrp)(phen)](**1**) | Hypochromism | 24.2% | $3.32 \times 10^5$ | ~8 | $0.66 \times 10^7$ |
| [Cu(saltrp)(bipy)](**2**) | Hypochromism | 12.0% | $3.10 \times 10^5$ | ~7 | $0.50 \times 10^7$ |
| [Cu(saltyr)(phen)](**3**) | Hypochromism | 20.0% | $3.47 \times 10^4$ | ~6 | $0.33 \times 10^6$ |
| [Cu(saltyr)(bipy)](**4**) | Hypochromism | 11.0% | $3.01\times 10^4$ | ~5 | $0.20 \times 10^6$ |

[a] Change in Absorbance; [b] Percentage of Hypochromism; [c] Intrinsic DNA binding constant [$M^{-1}$]; [d] $T_m$ of free DNA - $T_m$ of complex bound DNA; [e] Apparent DNA binding constant [$M^{-1}$] from fluorescence quenching study.

Comparison of $K_b$ of **1-4** revealed that complexes follow the order of **1**>**2**>**3**>**4** towards the DNA binding ability. Phenanthroline complexes **1** & **3** show a higher intercalative DNA binding ability than corresponding bipyridine complexes **2** & **4**. This can be attributed to the presence of extended aromatic ring of phen in **1** & **3**, making a non covalent interaction of the π system of the ligand with DNA base pairs more favourable and more effective than that of bipy ring in **2** & **4**.

Tryptophan schiff base complexes **1** & **2** show higher binding affinity than corresponding tyrosine schiff base complexes **3** & **4**. This can be explained based on the fact that tryptophan shows extended aromaticity over tyrosine which helps in effective stacking interaction of schiff base ligand with the DNA base pairs.

***B. Thermal Denaturation Study:***

Mode of complex binding with DNA via intercalation or external binding can be further confirmed by melting of DNA i.e thermal denaturation study. Melting temperature ($T_m$) represents the temperature at which a double helix is broken up into single stranded DNA. Interaction of molecules with DNA results in either increase or decrease of $T_m$ . While an increase in $T_m$ value is indicative of an intercalative binding, a decrease in $T_m$ value indicates base binding. The thermal denaturation profile of DNA in the absence and presence of **1-4** were monitored (**Figure 3**).

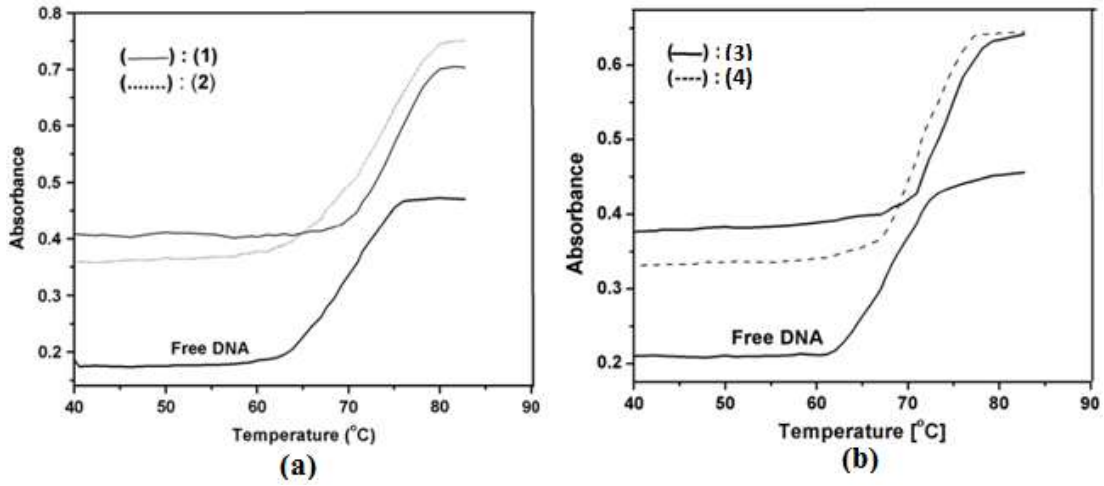


**Figure 3.** Thermal denaturation profiles of CT-DNA before and after addition of 1 & 2 (a); 3 & 4 (b). [Complex]= (60 μM). The DNA concentration was fixed at 60 μM.

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An increase of ~5-8ºC in the $T_m$ profile of DNA with complexes **1-4** was observed as compared to free DNA . This confirmed the intercalative binding of complexes with DNA and was in accordance with the results obtained from Absorption spectroscopy. The extent of increase in $T_m$ of DNA in the presence of complexes as compared to free DNA follows the order **1**>**2**>**3**>**4** (**Table 2**). This is in agreement with the order of DNA binding constants ($K_b$) for **1-4**, which confirms that the binding ability of complexes with the DNA follow the order **1**>**2**>**3**>**4**.

***C. Ethidium Bromide competitive binding by absorption spectroscopy:***

Intercalative binding was also demonstrated through competitive binding experiment using Ethidium Bromide (EB), a well known intercalator [37]. Free EB having an absorption maxima ~480nm, shifts to a higher wavelength followed by decrease in absorption on interaction with DNA. This is evidence of intercalation of EB into the DNA base stacks. Competitive binding study by addition of increasing amounts of **1-4** to the EB-DNA solution caused an enhancement in the absorption intensity which is an indication of competitive binding of **1-4** with EB to bind to DNA (**Figure 4** (**1** & **3**)). These results further confirmed the intercalative mode of binding of **1-4** with DNA.

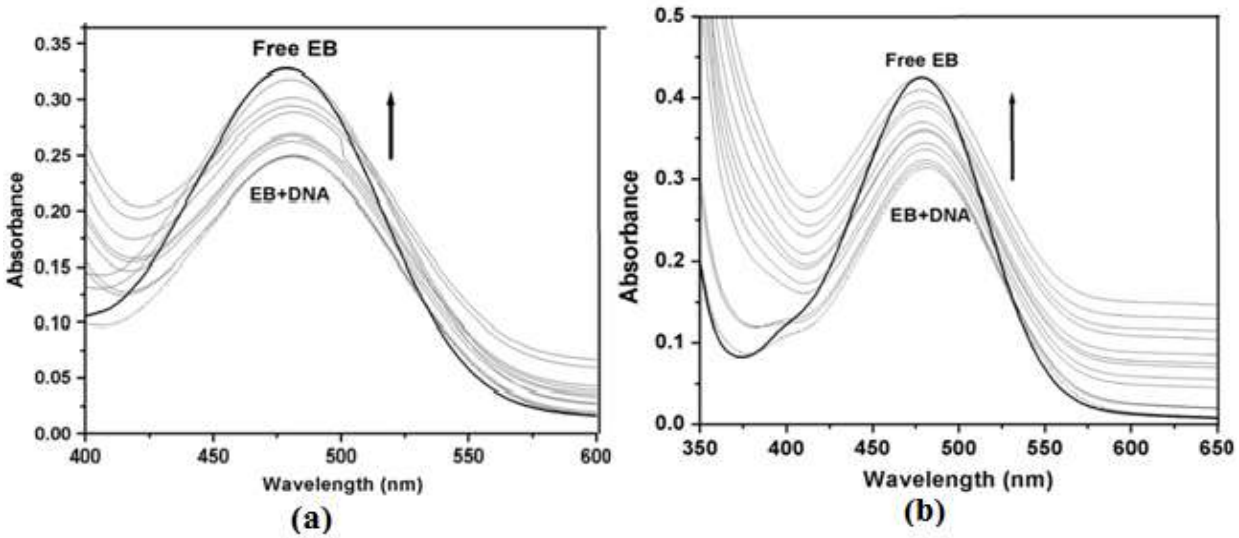


**Figure 4.** Absorption spectra of EB bound to DNA in the absence (·····) and presence ( — ) of increasing amounts of 1 (a) and 3 (b). Conditions: [EB] = 40 μM, [DNA] = 40 μM, [1] =(0–50 μM), [3] =(0–60 μM). . Arrow (↑) shows the absorbance changes upon increasing complex concentration.

***D. Viscosity Measurements:***

Interaction mode of **1-4** to DNA was also clarified by monitoring the change in viscosity of DNA upon addition of complexes which is sensitive to its length changes [38,39] . Complex binding by intercalation to DNA increases the viscosity of DNA, while it remains same for classical groove binding and decreases for non classical intercalation [40,41]. Viscosity measurements upon addition of increasing amounts of **1-4** (**Figure 5**) has shown a steady increase in viscosity of CT-DNA. Such behavior is consistent with other classical intercalators (i.e. EB) indicating that the complexes **1-4** intercalate between adjacent DNA base pairs, causing an increase in length of DNA helix thereby increasing the viscosity of DNA. Comparison of extent of viscosity increase of DNA clearly indicates that there is higher increase in viscosity on addition of **1** & **2** to CT-DNA when compared to addition of **3** & **4** to CT-DNA. The extent of increase in viscosity of DNA on addition of complexes follow the order **1**>**2**>**3**>**4** which is in accordance with the binding constants $K_b$ order and $\Delta T_m$ [**Table 2**] confirming that phen complexes **1** & **3** have better binding affinity than their corresponding bipy systems **2** & **4**. It may be also confirmed that trp schiff base complexes **1** & **2** bind strongly over their corresponding tyr schiff base complexes **3** & **4**.

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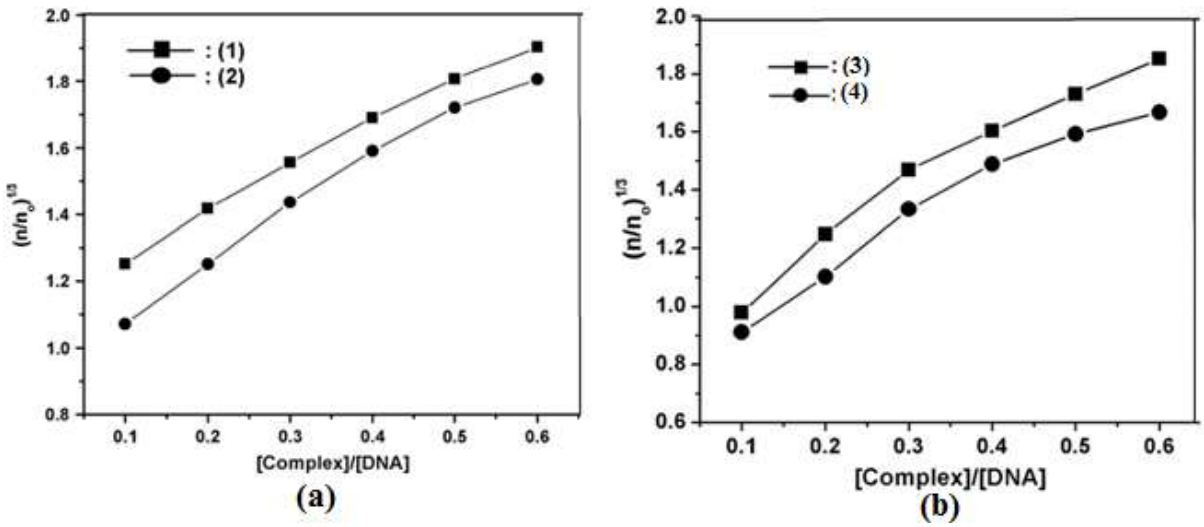


**Figure 5.** Effect of increasing amount of 1(■) and 2(●) (a); 3(■) and 4(●) (b) on the relative viscosities of CT-DNA at r.t. in 5 mM Tris–HCl buffer. Conditions: [DNA] = 200 μM, [complex] = 0– 120 μM.

***E. Ethidium Bromide competitive binding by Fluorescence Spectroscopy:***

To further clarify the intercalative interaction of complexes with DNA, competitive binding study with fluorescence quenching experiments based on displacement of the intercalating drug EB from EB-DNA system by **1-4** were performed and reported [28,29]. Free EB, displays a decrease in emission intensity in Tris HCl buffer medium because of quenching by solvent molecules. However EB strongly fluoresces in the presence of DNA due to complete intercalation between base pairs of DNA, a process that can be reversed by addition of competing molecule (fluorescence quenching) [42,43]. The fluorescence quenching and its extent of EB bound to DNA can be used to determine DNA binding mode and affinity of a given molecule. The fluorescence quenching curves of EB bound to DNA in the absence and presence of complexes were monitored (**Figure 6** (**1** & **3**)). The quenching plots indicate that the quenching of EB bound to DNA by complexes is in good agreement with the linear Stern-Volmer equation, which confirms that the complexes **1-4** bind to DNA. In the plot of $I_o/I$ versus [Complex]/[DNA], $K_{sq}$ is given by ratio of slope to intercept. The apparent binding constants ($K_{app}$) were reported as $0.66 \times 10^7$ $M^{-1}$, $0.50 \times 10^7$ $M^{-1}$, $0.33 \times 10^6$ $M^{-1}$ and $0.20 \times 10^6$ $M^{-1}$ for **1-4** resp, using the equation $K_{EB}[EB]=K_{app}[complex]$, where the complex concentration was the value at 50% reduction of fluorescence intensity of EB and $K_{EB}$ is $1.0 \times 10^7$ $M^{-1}$ . The closeness in the $K_{app}$ values of **1-4** compared to $K_{EB}$ value suggest that **1-4** are as good intercalators as EB and the order of binding propensity of complexes **1**>**2**>**3**>**4** can be further confirmed by the order of $K_{app}$ values for **1-4** (**Table 2**).

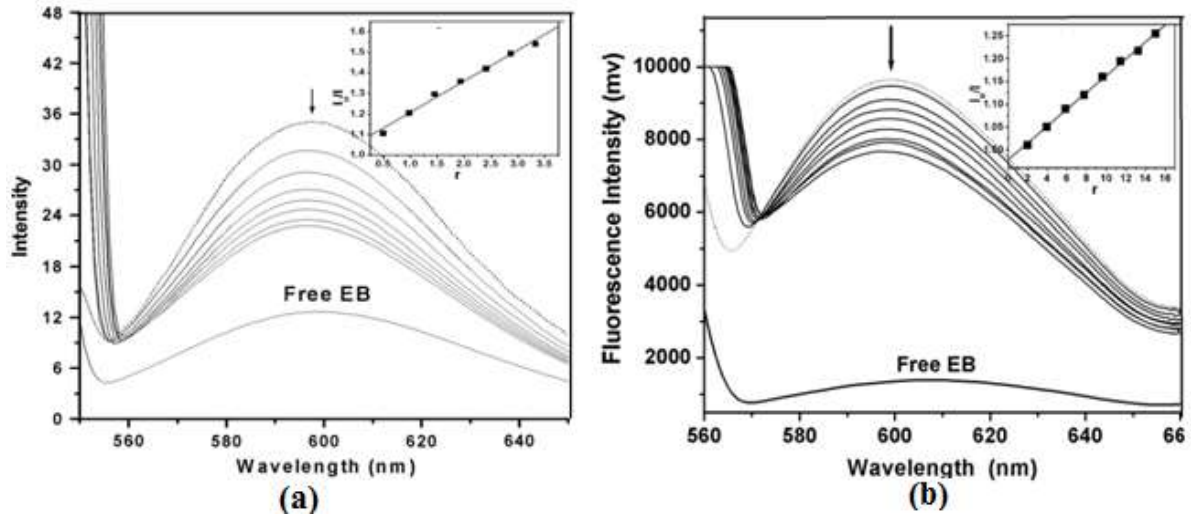


**Figure 6.** Emission spectra of EB bound to DNA in the absence (·····) & presence ( — ) of increasing amounts of 1 (a) and 3 (b). Inset: Stern–Volmer quenching curves.

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Fluorescence Scatchard plots for the binding of EB to CT-DNA in the presence of complexes were obtained as reported earlier by Lepecq and Paoletti. The binding isotherms of EB and DNA in the presence of **1**-**4** (**Figure 7** (**1** & **3**)) displayed a decrease in slope with no change in the intercept (Type B behaviour) indicating an intercalative mode of binding of the complexes to DNA. These results also concur with those obtained from other DNA binding studies i.e. absorption spectroscopy, Thermal denaturation, viscosity measurements and EB competitive binding by absorption and emission spectral studies.

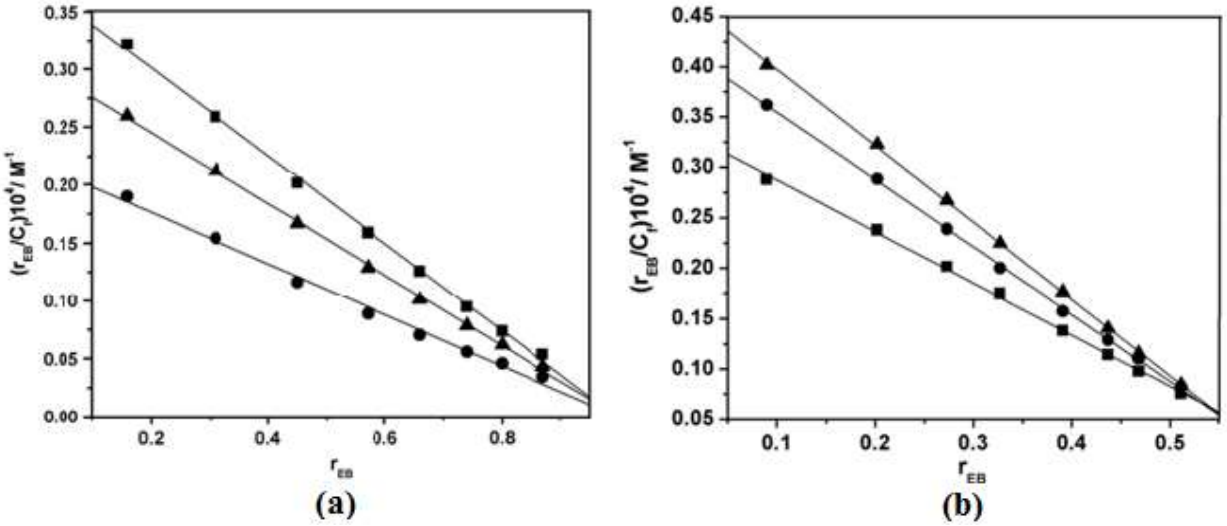


Figure 7. Fluorescence *Scatchard* plots for the EB bound to CT-DNA (a) in the absence (■) and presence of 1(●) and 2(▲) ; (b) in the absence (▲) and presence of 3(■) and 4(●) .The term $r_{EB}$ is the concentration ratio of bound EB to total DNA and $c_f$ is the concentration of free EB.

## V. CONCLUSIONS AND FUTURE SCOPE

In recent years studies of metal-DNA interaction have received considerable interest for the development of artificial endonucleases and anticancer drug therapies. Owing to this, in this article we have presented a comparison of DNA binding interaction of Cu(II) amino acid schiff base complexes with heterocyclic bases reported from our laboratory. Cu(II) complexes **1**-**4** with ONO donor schiff base (saltrp, saltyr) and NN donor heterocyclic bases (phen, bipy) were synthesized and characterized. DNA binding study by various techniques revealed that complexes bind to DNA by intercalative mode and binding affinity follows the order of **1**>**2**>**3**>**4**. Tryptophan schiff base complexes display a higher DNA binding ability over tyrosine schiff base complexes due to the extended ring size of amino acid i.e trp which enhances stacking interaction with DNA. Further prospects in this area lie in the development of chemical nucleases with new Schiff bases which are capable of achieving good DNA binding which is a prerequisite for nucleases.

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