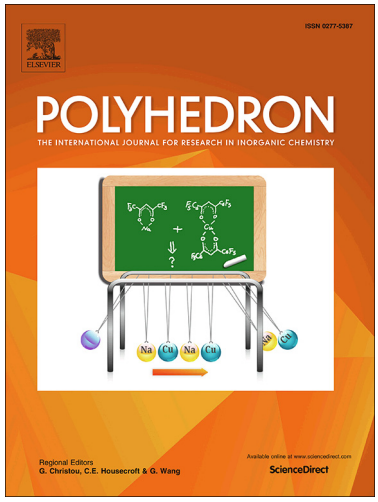

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Article

DNA/BSA binding studies of peripherally tetra substituted neutral azophenoxy zinc phthalocyanine

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Abstract

A tetra substituted neutral zinc phthalocyanine (ZnPc-4) bearing 4-phenylazophenoxy group at the periphery had been prepared from zinc acetate dihydrate $Zn(CH_3COO)_2.2H_2O$ and 4-(phenylazophenoxy)phthalonitrile. The interaction of neutral metallophthalocyanine, ZnPc-4 with Calf Thymus (CT) DNA was investigated using absorption titrations, competitive Fluorescent Intercalator Displacement (FID) assay, minor groove binding assay and viscosity measurement. The binding studies revealed a probable intercalative mode of interaction of ZnPc-4 with DNA with an appreciable binding constant. The bovine serum albumin (BSA) binding activity of ZnPc-4 was evaluated by fluorescence titration.

Keywords: ZnPc-4, CT-DNA, Fluorescent Intercalator Displacement (FID) assay, binding constant, BSA

1. Introduction

Phthalocyanines (Pcs), which are conventionally used as dyes and pigments[1], are a class of macrocyclics of great interest with wide range of applications. Studies on the interactions of transition metal complexes with nucleic acids have gained considerable momentum in the recent past with an aim to develop new DNA molecular probes and novel chemotherapeutic agents[2,3]. Metallophthalocyanines (MPcs) are well known for their use as medicinal agents and as DNA interacting probes, on account of their large π delocalized planar surface area, which can be easily modified according to the biological target[4–6]. However, the broad applicability of unsubstituted phthalocyanines can be severely hampered by their insolubility in common organic solvents and in aqueous media[7,8].This problem can be solved by the judicious introduction of electron-donating substituents (alkyl, alkoxy, alkylthio chains, or bulky phenyl, aryl groups), electron- withdrawing substituents (chloro, bromo, or nitro moieties), polar ionizable groups (anionic sulfonyl, carboxyl or cationic amino or quaternized ammonium groups) and neutral biomolecules like glucose, lactose, galactose etc. at the peripheral and non-peripheral positions[9–16]. A majority of the reported DNA targeting Pcs were observed to be cationic, while very few of them were observed to be anionic in nature. Charged Pcs were found to be aqueous soluble, which was

essential for biological applications. Cationic Pcs have an inclination towards the anionic phosphodiester backbone of DNA, thus improving the cell permeability and the cleaving effect of cationic phthalocyanines[17–19]. This prompted us to study the behavior of neutral metallophthalocyanine in a similar environment, and to the best of our knowledge, very few reports are available on the DNA interaction studies of neutral MPcs to date. Arslantas et al. have focused on the DNA binding interaction of various neutral MPcs peripherally substituted with functional groups such as 4-(4-hydroxyphenyl)-1-phenylethyl) phenoxy group, 3,4,5-trimethoxybenzyloxy substituents, dimethyl- 5-(phenoxy)-isophthalate, 5-tert-butyl-2-hydroxyphenoxy groups etc [20–23]. In another report, Durantini et al., had studied the photodynamic application of zinc phthalocyanine peripherally substituted with methoxy and trifluoromethylbenzyloxy substituents[24].

The photo switching property of azobenzene based compounds often limit their biological applications, since the strong scattering tendency of UV light reduces the effective penetration of cells and tissues [25]. Moreover, UV light can trigger unwanted cellular responses like apoptosis, which can further complicate the studies[26,27]. However, a few reports had described the use of azobenzene in conjugation with pharmacologically active compounds of high therapeutic index[28,29] and Zhang et al. in another report had evaluated the photo dependent antibacterial activity of honeycomb patterned dodecyloxy-azo-zinc phthalocyanine film (daZnPc), towards *Escherichia coli (E. coli)*[30]. BSA and human serum albumin (HSA) are blood plasma proteins, which play critical role in the transport of drugs to specific targets in the blood, and their distribution and metabolism in the body[31,32]. So the BSA-drug interaction has vital importance in the formulation and the production of novel drug.

In this perspective, we hereby report the DNA/BSA binding studies of zinc phthalocyanine (ZnPc-4) peripherally substituted with 4-phenylazophenol moiety by oxygen linkage. The mode of interaction of ZnPc-4 with CT-DNA had been investigated using electronic absorption titrations, fluorescent intercalator displacement assays, minor groove binding assays and viscosity change studies. The BSA binding affinity of ZnPc-4 was evaluated by fluorescence spectroscopy.

2. Experimental

Materials & Instrumentation

CT-DNA and bio-reagent grade lyophilized powder of BSA were purchased from Sigma-Aldrich. The commercially purchased chemicals were used without further purification. UV-visible absorption spectra were recorded on a Schimadzu UV-Visible 2600 Spectrophotometer with quartz cuvette and fluorescence spectra were obtained from Cary-Eclipse Agilent fluorescence spectrophotometer. The viscosity measurements were taken using a 15 ml Ostwald Viscometer.

3. Results and discussion

3.1. Preparation of zinc phthalocyanine, ZnPc-4

The molecular structure of the compound under study, 2,9,16,23-Tetra-(4-phenylazophenoxy) zinc(II)phthalocyanine (**ZnPc-4**) is represented in Figure 1.

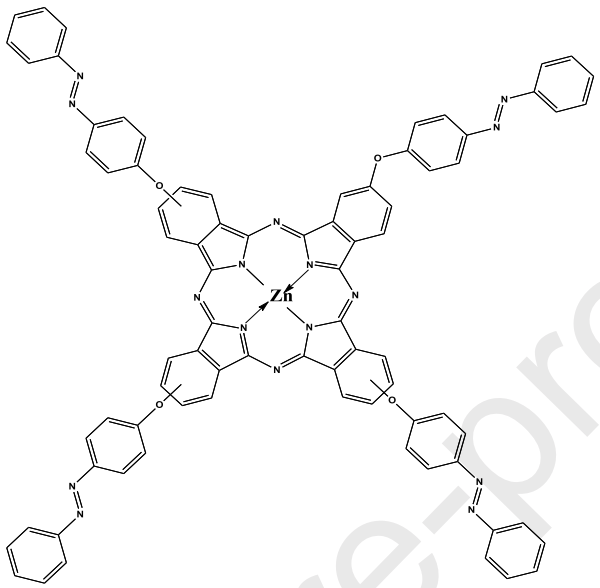

Figure 1. Molecular structure of 2,9,16,23-Tetra-(4-phenylazophenoxy) zinc(II)phthalocyanine, ZnPc-4

This tetra substituted zinc phthalocyanine was prepared by cyclotetramerization of 4-(4-(phenylazophenoxy) phthalonitrile by refluxing with $Zn(CH_3COO)_2.2H_2O$ in n-pentanol/DBU at 170°C under N_2 atmosphere. The compound was fully characterized with electronic, FT-IR, ^{1}HNMR and mass spectroscopic techniques and the results were concordant with the expected structure. The synthesis and characterization details had been reported elsewhere[33] and additional data are given in Supplementary Information (SI).

3.2. Interaction of ZnPc-4 with duplex DNA

3.2.1. Determination of binding of ZnPc-4 to CT-DNA using absorption titrations

The UV-visible absorption titration of ZnPc-4 with CT-DNA was conducted at room temperature in a 50mM Tris-HCl buffer containing 50mM NaCl (pH 7.4). DNA was dissolved in the buffer solution by ultra-sonication. UV absorbance of DNA stock solution in buffer at 260 and 280 nm was measured and the ratio A_{260}/A_{280} was found to be 1.87, ensuring the DNA sample was sufficiently free of protein. The concentrations of DNA per nucleotide phosphate was calculated from the absorbance at 260 nm using the average extinction coefficient value of ε_{260} = 6600 $M^{-1}cm^{-1}$. DNA was stored at 4°C overnight and was used within 4 days[34]. The stock solution of ZnPc-4 was prepared in DMF and then diluted to the desired concentration using Tris-HCl buffer of pH 7.4. The absorption titrations were carried out by

adding increasing amounts of DNA, i.e., 1µM aliquots, to a fixed concentration (10µM) of ZnPc-4 contained in a quartz cell, with phosphate/drug (p/d) ratio ranging from 0.1 to nearly 1.0. Absorption spectra were recorded after each addition.

Binding constant was calculated by plotting [DNA] in the x-axis and [DNA]/ε_a-ε_f in the y-axis, where ε_a was extinction coefficient of bound complexes and ε_f was the absorption coefficient of free complexes. From the absorption titration data, the binding constant was determined using Wolfe-Schimer equation **(1)** [35]:

$$\frac{[\mathbf{DNA}]}{\boldsymbol{\varepsilon_a - \varepsilon_f}} = \frac{[\mathbf{DNA}]}{\boldsymbol{\varepsilon_b - \varepsilon_f}} + \frac{\mathbf{1}}{\mathbf{K_b(\varepsilon_b - \varepsilon_f)}} \qquad \textbf{(1)}$$

where [DNA] was the concentration of DNA in base pairs and the apparent absorption coefficients, ε_a, ε_f, and ε_b corresponded to A_{obsd}/[M], the extinction coefficient of the free compounds and the extinction coefficient of the compound when fully bound to DNA respectively. A linear fitting of the plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] was done and the binding constant K_b was given by the ratio of slope to the intercept.

With increased addition of CT-DNA, the absorbance of ZnPc-4 got decreased, reaching saturation beyond the DNA concentration of 10 µM. Interaction of ZnPc-4 with CT DNA was observed by the hypochromism of three major bands located at λ_{max} of 350, 640 and 686 nm associated with slight red shift for each band. As shown in Figure 2 , ZnPc-4 exhibited hypochromism of about 26%, 20.8%, 18.73% and bathochromic shift of about 2.5 nm, 1.5 nm, 2 nm for the bands situated at 350, 640, 686 nm respectively. Three isosbestic points were also seen at 720, 578 and 442 nm on the addition of increasing concentrations of CT DNA. Hypochromism associated with a slight bathochromic shift usually resulted from the intercalative mode of binding, involving a stacking interaction between an aromatic chromophore and the base pairs of DNA[12]. The highly planar central moiety of ZnPc-4 with the conjugated ring systems, could possibly incorporate an intercalation mode of binding with CT DNA. The binding constant, K_b value was calculated as 1.58 x10^5 M^{-1}.

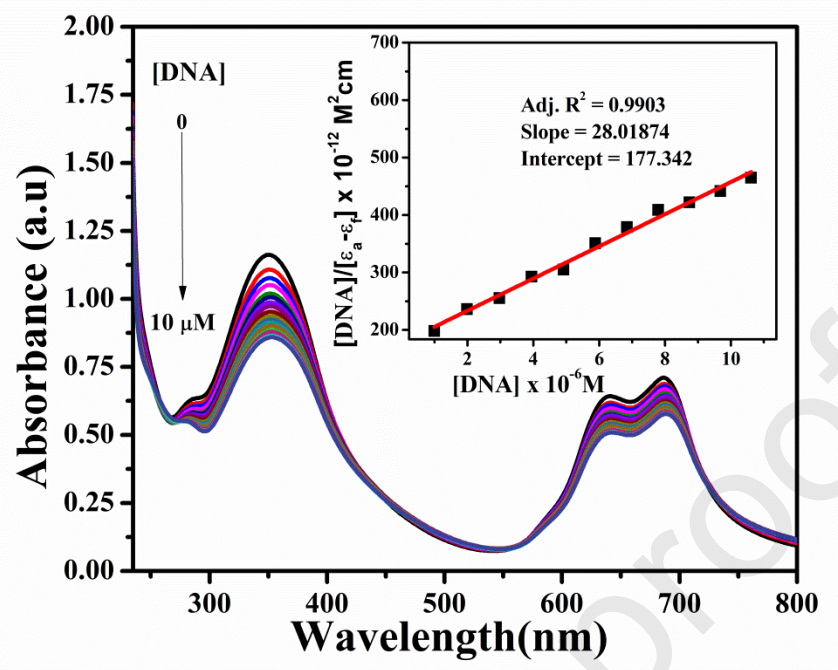

Figure 2. Absorption spectra of ZnPc-4 in DMF/Tris-HCl buffer upon addition of CT DNA Inset: plots of $[DNA]/\varepsilon_a-\varepsilon_f$ verses [DNA] for the titration of DNA with ZnPc-4.

3.2.2. Fluorescent Intercalator Displacement (FID) Assay

FID assay is a non-destructive technique which relies on the change in fluorescence observed on the displacement of DNA-bound ethidium bromide (EB) by another DNA binding compound. EB is a classical DNA intercalator, as it binds to the biomolecule by occupying the intercalative sites between DNA bases and the fluorescence yield of EB is significantly enhanced on DNA binding. The ability of the compound under study to displace EB from the intercalative sites on DNA can be monitored by a decrease in EB–DNA fluorescence where the amount of unbound or free EB would be increased[36]. Although the present compound, ZnPc-4 was inherently fluorescent with λ_{em} situated at 694 nm (given in SI S9), its fluorescence got quenched due to aggregation in aqueous/buffer medium[37]. Hence EB displacement assay had been performed to find out the mode of interaction of compound with DNA. To study the competitive binding of EB with ZnPc-4, 10µM CT DNA was pretreated with EB for 10 minutes at room temperature in Tris-HCl buffer of pH 7.4 and excited at 480 nm and the emission spectra were recorded in the absence, and at various amounts of ZnPc-4 in DMF, up to the ratio of [DNA]/[ZnPc-4] was reached to unity.

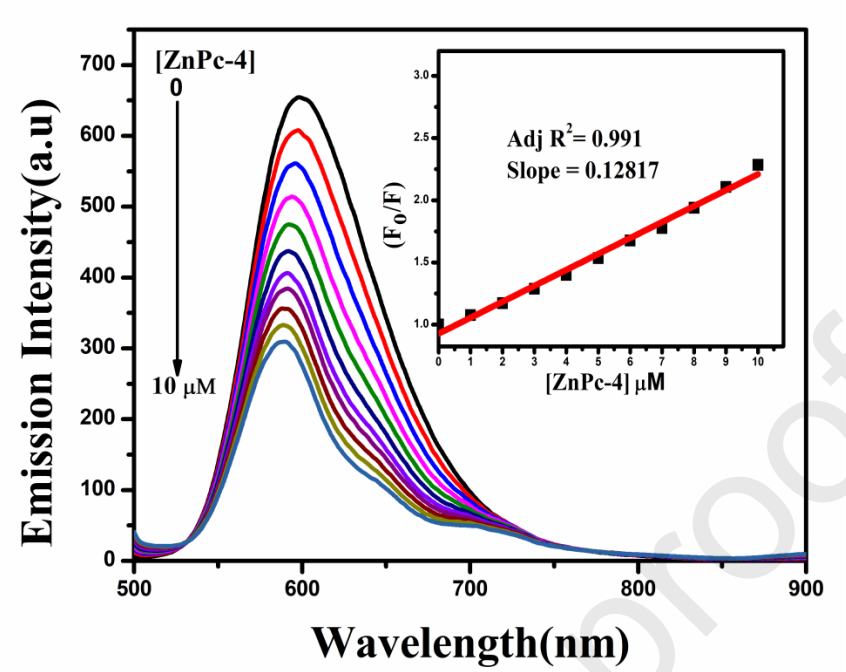

Figure 3. Competitive Intercalator displacement assays: Fluorescence titration of the EB–CT DNA complex with ZnPc-4. The EB–CT DNA complex was excited at 480 nm and emission spectra were recorded from 500–800 nm. Inset: Stern-Volmer plots of F_o/F verses [ZnPc-4]x10^{-6} M for the titration of CT DNA with ZnPc-4.

Figure 3 shows the emission spectra of EB-DNA adducts (λ_{ex}=480 nm; λ_{em}=598 nm) in the presence of increasing amounts of ZnPc-4 in DMF. The emission intensity of EB-DNA adduct was gradually decreased on the addition of ZnPc-4 and the extend of quenching was quantitatively estimated by using the Stern–Volmer (S-V)equation **(2)** [38],

$$\frac{F_o}{F} = 1 + K_{SV}[Q] \qquad (2)$$

where F_o and F were the fluorescence intensities in the absence and presence of the quencher ZnPc-4, K_{SV} is the S-V quenching constant and [Q] the quencher concentration. The quenching constant K_{SV} was obtained as 1.282 x 10^5 M^{-1} from the slope of the S-V plot F_o/F versus [Q] given in the inset of Figure 3.

A linear S-V plot as obtained in the inset of Figure 3 suggested only one type of binding or quenching process, either static or dynamic quenching, which can be differentiated using equation **(3)**.

$$K_{SV} = K_q X \tau_o \qquad (3)$$

The approximate quenching constant (K_q) can be calculated from the equation **(3)**, where τ_o is the lifetime of CT DNA-EB adduct was reported as 19.2 ns in the absence of the complex[39]. The calculated value of K_q (6.67x10^{12} M^{-1} S^{-1}) was in the order of 10^{12} M^{-1} S^{-1}, which was 100 times larger than the maximum diffusion collision quenching constant (2 x10^{10} M^{-1} S^{-1}) of a variety of quenchers with

biomacromolecules[40]. The result unambiguously showed the existence of a static quenching mechanism rather than dynamic collision. This pointed out that the gradual formation of non-fluorescent DNA-Pc adducts by replacing EB from the binding site could be the reason for the static quenching, rather than the collision deactivation process.

$$\boldsymbol{K_{app} = \frac{K_{EB}\,[EB]}{[Q]}} \qquad \textbf{(4)}$$

K_{app} can be defined as the binding constant at which 50% reduction in fluorescence intensity of DNA-EB adduct occurs with the addition of quencher. The apparent binding constant[41] K_{app} was calculated as 3.64 x10^6 M^{-1} using the equation **(4),** where K_{EB} was the binding constant of EB (1x10^7 M^{-1}), while [EB] and [Q] were the concentrations of EB and quencher ZnPc-4 respectively at 50% reduction in fluorescence intensity.

3.2.3. Minor groove binder displacement assay

Minor groove binder displacement assay with another classical groove binder Hoechst 33258 was performed, in order to investigate the possibility of groove binding mode of interaction of ZnPc-4 with CT DNA. Hoechst 33258 dye (HD) is chemically 2′-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5′-bi-1*H*-benzimidazole trihydrochloride hydrate and is known to be a potential minor groove binder, which shows great affinity towards A-T (adenine-thymine) rich sequence of DNA double helix. Like EB displacement assay, a similar fluorescence competition experiment was carried out using HD, as shown in Figure 4. Initially the HD-DNA binding occurred in the minor groove of DNA double helix. The fluorescence quantum yield of HD increased significantly on interacting with DNA. The displacement of bound HD from its binding site on CT DNA was implicated from a decrease in its fluorescence intensity on addition of the compound.

The apparent binding constant K_{app} was calculated as 1.27 x10^5 M^{-1} using the equation **(4),** where K_{HD} was the binding constant of HD (9.6x10^5 M^{-1})[42], while [HD] and [Q] were concentrations of HD and quencher ZnPc-4 respectively at 50% reduction in fluorescence intensity.

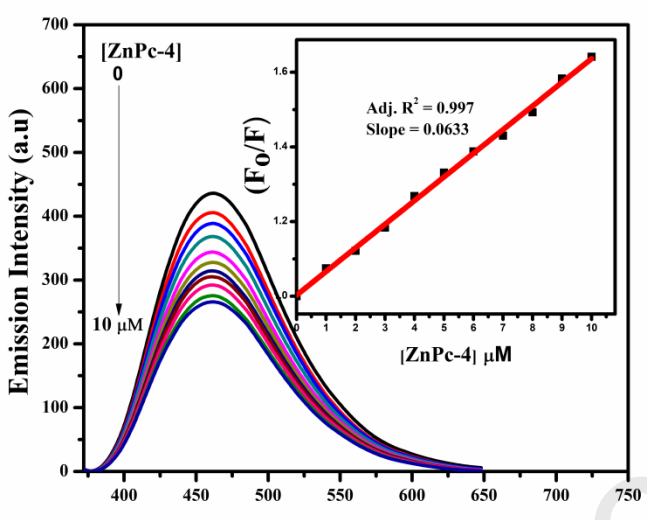

Figure 4. Competitive Minor groove binder displacement assays: Fluorescence titration of the HD-CT DNA complex with ZnPc-4. HD-CT DNA complex was excited at 355 nm and emission spectra were recorded from 370–650 nm. Inset: Stern-Volmer plots of F_o/F verses [ZnPc-4]x10^{-6} M for the titration of CT DNA with ZnPc-4.

Table 1. Comparison of K_{SV} and K_{app} value of ZnPc-4 with Fluorophore-DNA bound adduct to predict the mode of interaction of DNA binding for [phosphate or DNA]/[drug or compound]([p]/[d]) ratio ranges from 0-1 with 0.1 increment

SI No.	Type binding assay	[p]/[d] ratio	Linear fit correlation coefficient Adj.R^2	K_{SV} (M^{-1})	Apparent binding constant (K_{app}) (M^{-1})
1	EB displacement assay	0-1 (0.1 increment)	0.991	$1.28x10^5$	$3.64 x10^6$
2	HD displacement assay	0-1 (0.1 increment)	0.997	$0.663x10^5$	$1.27 x10^5$

From the Table 1 given above, the quantitatively determined parameters clearly suggested that the compound ZnPc-4 was presumably interacting with DNA via an intercalative mode of interaction.

3.2.4. Viscosity measurements

Hydrodynamic measurements are considered as the unambiguous and the most reliable test to elucidate the strength and mode of DNA-drug interactions, unless the crystallographic data of DNA bound drug conjugate are available. When a molecule interacts with DNA by intercalative interactions, it can cause a significant increase in the viscosity of a DNA solution, since the insertion of intercalator causes separation of base pairs, and hence, an increase in the overall DNA molecular length. On the other

hand, non-classical or partial intercalators, such as the $[Ru(phen)_3]^{2+}$ cation usually bend or kink the DNA helix, decreasing the viscosity of DNA[43,44].

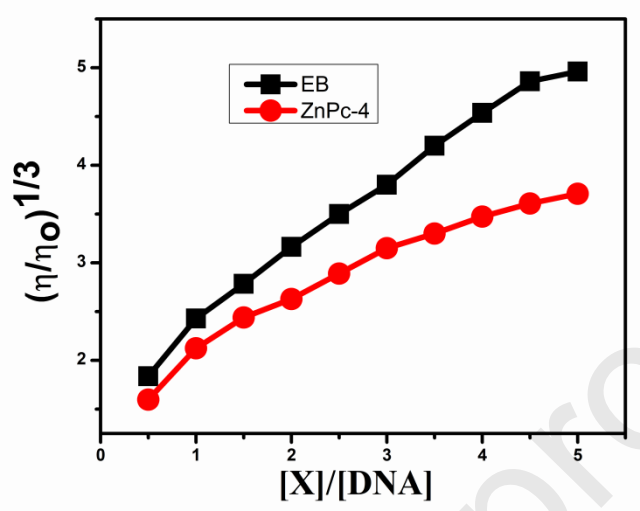

Figure 5. Effect of increasing amount of ZnPc-4 on the relative viscosity of CT DNA in 50 mM Tris-HCl buffer.

Viscosity measurements were carried out at room temperature using an Oswald viscometer of 15 ml capacity. The DNA was sonicated for 10 min and bubbled with nitrogen, for 3 minutes for homogenous mixing. The DNA concentration was kept constant and the concentration of ZnPc-4 was increased from 0 to 10 µM in different samples. The flow time was measured three times for each sample, viscosity and $(\eta/\eta_o)^{1/3}$ were calculated for each and every trial and finally an average $(\eta/\eta_o)^{1/3}$ was calculated. Data were presented as $(\eta/\eta_o)^{1/3}$ versus the ratio of [X]/[DNA], where η is the viscosity of DNA in the presence of the complex, η_o is the viscosity of DNA alone and [X] is the concentrations of either EB or ZnPc-4. Viscosity values were calculated from the observed flow time of DNA-complex solutions corrected for the flow time (t^o) of buffer alone, i.e., $\eta = t\text{-}t^o$. In the same way, the viscometric titration of EB was also performed with CT DNA and cross plotted with the viscometric response curve of DNA-ZnPc-4. As shown from Figure 5, the viscosity of DNA solution increased steadily with the addition of ZnPc-4 aliquots, mostly due to the hydrophobic core insertion of the phthalocyanine molecules between the DNA base pairs, resulting in the lengthening of DNA helix[45].

3.3. BSA binding experiment of ZnPc-4

BSA-drug interaction has a critical role in pharmacokinetic and pharmacodynamic phases of drug metabolism. When a compound is administered as a drug, it would immediately encounter blood plasma proteins BSA and HSA in the blood. BSA displays a significant contribution to the physiological functions and effective delivery of drug to specific site, hence the investigation on the interaction of drugs/compound with BSA is of great interest. The binding of ZnPc-4 to BSA was studied by

fluorescence spectroscopy in 50mM Tris HCl buffer at room temperature. The fluorescence of BSA at 348 nm was mainly attributable to the amino acid tryptophan in the macromolecule[32].

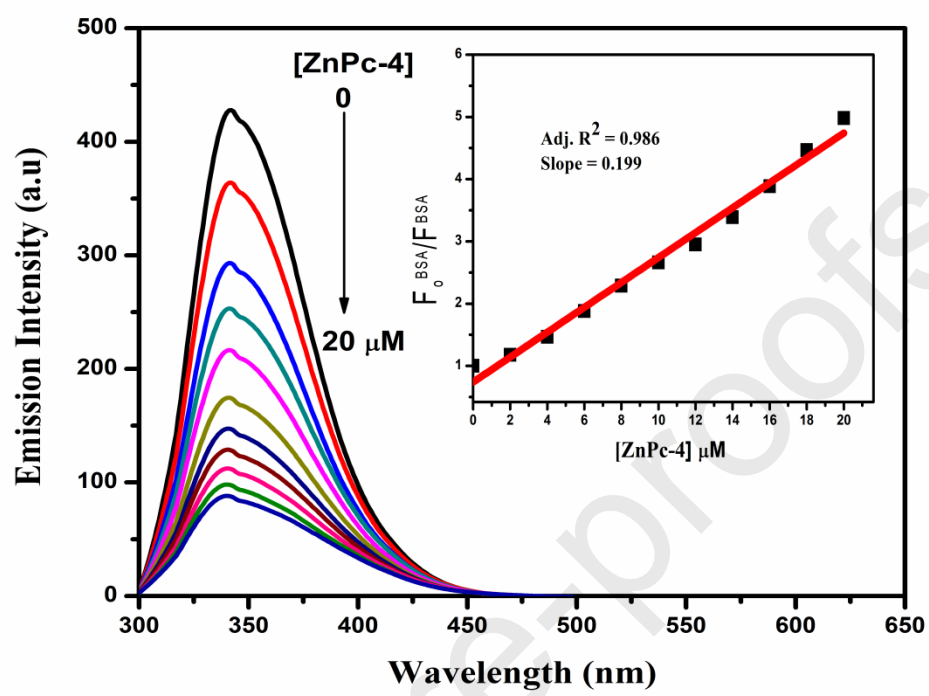

Figure 6. Fluorescence spectral changes of BSA on addition of varying concentrations of ZnPc-4 in buffer. [BSA] = 27.28×10^{-6} M, [ZnPc-4] varies from 0 to 20×10^{-6} M. Excitation wavelength = 280 nm.

Figure 6 shows the fluorescence emission spectra of BSA in the presence of different concentrations of ZnPc-4. The emission intensity of BSA was found to be diminished with the addition of ZnPc-4, and this observation suggested that the compound ZnPc-4 was interacting with BSA at the proximity of tryptophan residues and the extend of interaction can be quantified by determining the S-V quenching constants (K_{SV}^{BSA}) from equation **(4)**.

As discussed in section 3.2.2., the linear S-V plot revealed that either static or dynamic quenching process was taking place. Likewise the slope K_{SV}^{BSA} of S-V plot (inset of Figure 6) was calculated as $1.99\text{x}10^{5}$ M^{-1}. The bimolecular quenching constant K_q of the BSA-complex adduct was estimated using the equation **(3)**; where τ^{BSA}, the approximate lifetime of fluorophore BSA in the absence of quencher was usually taken as 10 ns[46,47]. The K_q value was estimated as $1.99\text{x}10^{13}$ $M^{-1}s^{-1}$, which was quite higher than the proposed value of the order of 10^{10} $M^{-1}s^{-1}$ for diffusion controlled dynamic quenching, and hence it can be assumed that the BSA-ZnPc-4 interaction obeyed static model quenching process. The decrease in the intrinsic fluorescence intensity of tryptophan with increasing concentration of ZnPc-4 indicated that the compound readily bound to BSA by the approaching the tryptophan enriched subdomains of BSA. This also suggested that the primary binding sites of these molecules were very close to tryptophan residues, since the occurrence of quenching requires molecular contact as per the static quenching model[48].

4. Conclusions

In summary, DNA/BSA binding activity of the compound peripherally tetra-(4-phenylazophenoxy) substituted zinc phthalocyanine ZnPc-4 was evaluated by different instrumental techniques. The DNA binding studies of ZnPc-4 towards CT DNA was carried out in 50mM Tris-HCl-NaCl buffer of pH 7.4 by UV-visible absorption titrations, competitive fluorescence quenching titration and viscosity measurements. The competitive fluorescent displacement assay was carried out with the classical intercalator Ethidium bromide and minor grove binder Hoechst dye and binding mode was predicted by comparing their S-V quenching constant K_{SV} and apparent binding constant (K_{app}). The value of K_{SV} 1.28 x 10^5 M^{-1} and K_{app} was higher for EB displacement assay than HD displacement assay. The intrinsic binding constant K_b from absorption titration was 1.58 x 10^5 M^{-1} and the trend of relative viscosity of DNA solution was increasing with increase in [ZnPc-4]/[DNA] ratio, and all these observations clearly pointed out that ZnPc-4 was interacting with DNA via intercalative mode. The BSA interaction experiment revealed that the bimolecular quenching constant K_q was of the order of 10^{13} $M^{-1}s^{-1}$, so the binding interaction of ZnPc-4 with BSA was assumed to be following static fluorescence quenching mechanism. The strong binding interaction of ZnPc-4 with BSA suggested that it can be easily transported in the blood, hence the synthesized molecule ZnPc-4 can be considered as a potential candidate for further studies and optimizations as a drug.

Conflict of interest:

The authors declare no conflict of interest.

Acknowledgements

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Figures and Tables

DNA/BSA binding studies of peripherally tetra substituted neutral azophenoxy zinc phthalocyanine

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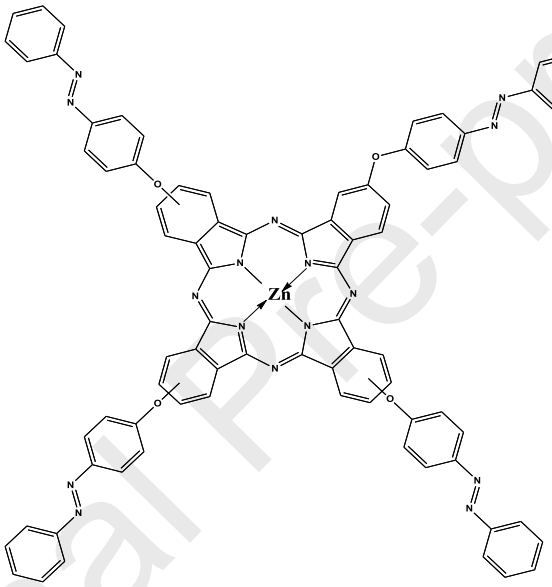

Figure 7. Molecular structure of 2,9,16,23-Tetra-(4-phenylazophenoxy) zinc(II)phthalocyanine, ZnPc-4

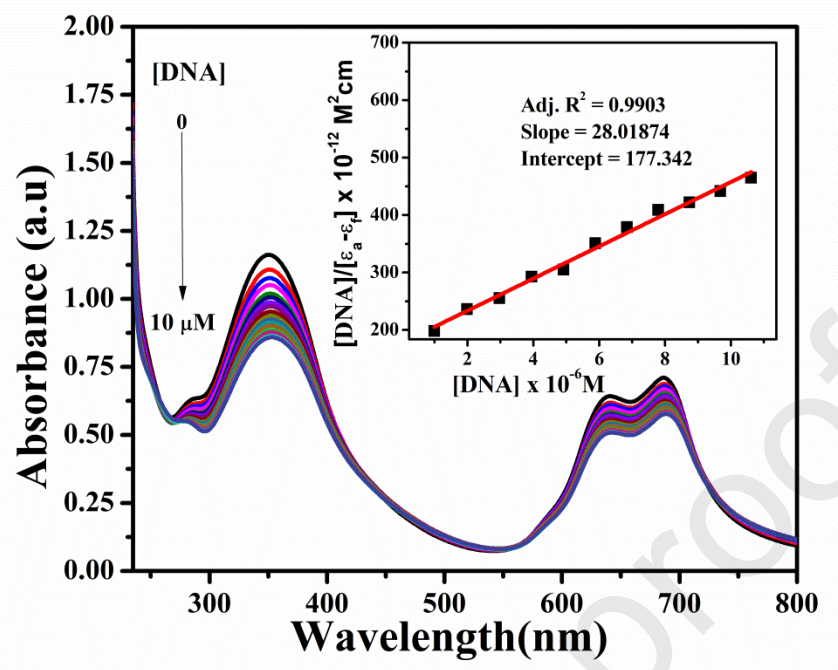

Figure 8. Absorption spectra of ZnPc-4 in DMF/Tris-HCl buffer upon addition of CT DNA Inset: plots of $[DNA]/\varepsilon_a-\varepsilon_f$ verses [DNA] for the titration of DNA with ZnPc-4.

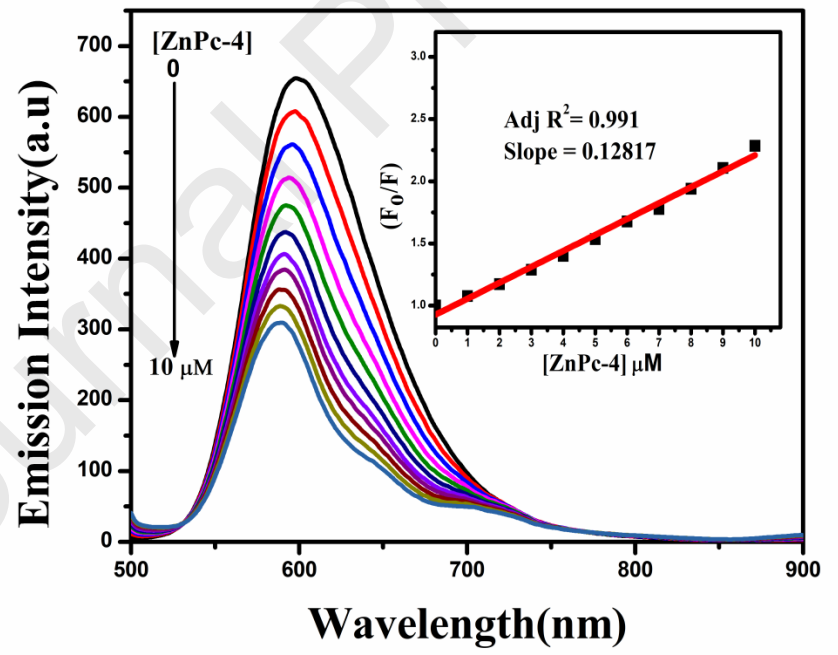

Figure 9. Competitive Intercalator displacement assays: Fluorescence titration of the EB–CT DNA complex with ZnPc-4. The EB–CT DNA complex was excited at 480 nm and emission spectra were recorded from 500–800 nm. Inset: Stern-Volmer plots of F_o/F verses $[ZnPc-4]x10^{-6}$ M for the titration of CT DNA with ZnPc-4.

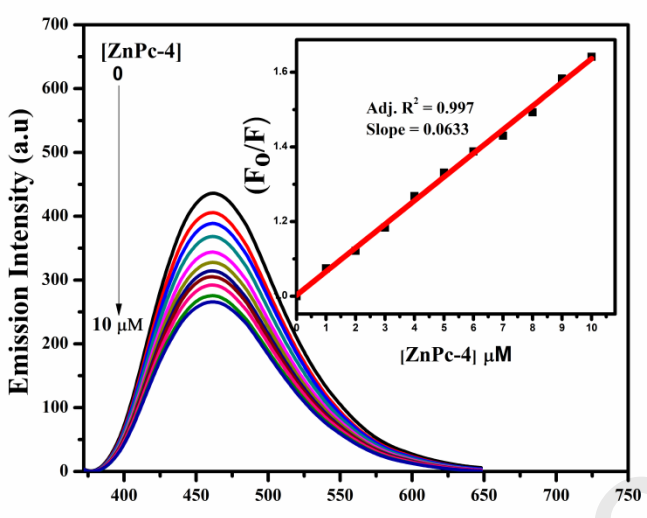

Figure 10. Competitive Minor groove binder displacement assays: Fluorescence titration of the HD-CT DNA complex with ZnPc-4. HD-CT DNA complex was excited at 355 nm and emission spectra were recorded from 370–650 nm. Inset: Stern-Volmer plots of F_o/F verses [ZnPc-4]x10^{-6} M for the titration of CT DNA with ZnPc-4.

Table 2. Comparison of K_{SV} and K_{app} value of ZnPc-4 with Fluorophore-DNA bound adduct to predict the mode of interaction of DNA binding for [phosphate or DNA]/[drug or compound]([p]/[d]) ratio ranges from 0-1 with 0.1 increment

SI No.	Type binding assay	[p]/[d] ratio	Linear fit correlation coefficient Adj.R^2	K_{SV} (M^{-1})	Apparent binding constant (K_{app}) (M^{-1})
1	EB displacement assay	0-1 (0.1 increment)	0.991	$1.28x10^5$	$3.64 x10^6$
2	HD displacement assay	0-1 (0.1 increment)	0.997	$0.663x10^5$	$1.27 x10^5$

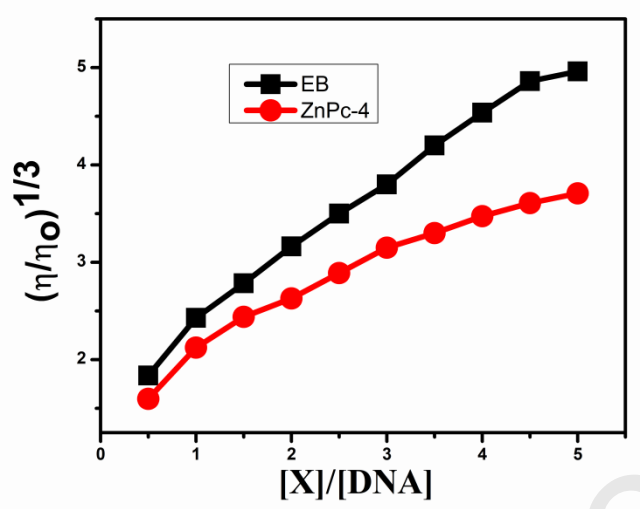

Figure 11. Effect of increasing amount of ZnPc-4 on the relative viscosity of CT DNA in 50 mM Tris-HCl buffer.

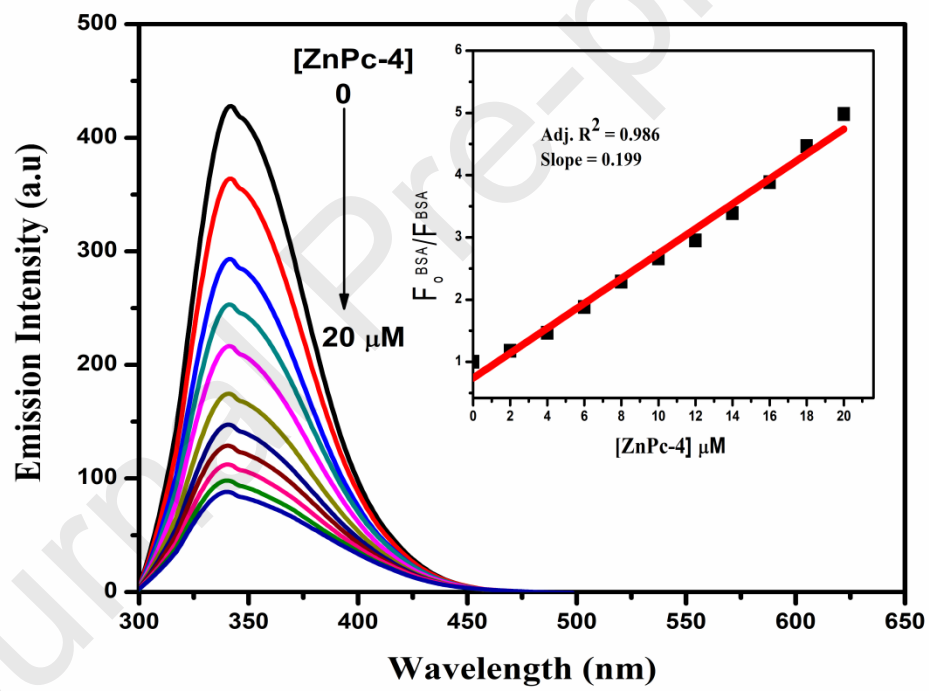

Figure 12. Fluorescence spectral changes of BSA on addition of varying concentrations of ZnPc-4 in buffer. [BSA] = 27.28×10^{-6} M, [ZnPc-4] varies from 0 to 20×10^{-6} M. Excitation wavelength = 280 nm.

- Synthesized peripherally 4-phenylazophenoxy substituted zinc phthalocyanine (ZnPc-4)
- DNA binding affinity of ZnPc-4 was studied by UV-visible, fluorescence titrations and viscosity measurements
- ZnPc-4 show intercalative mode of interaction with DNA by an appreciable binding constant of the order of 10^5 M^{-1}
- Static interaction of ZnPc-4 with BSA was investigated by fluorescence titration

Graphical abstract

DNA/BSA binding studies of peripherally tetra substituted neutral azophenoxy zinc phthalocyanine

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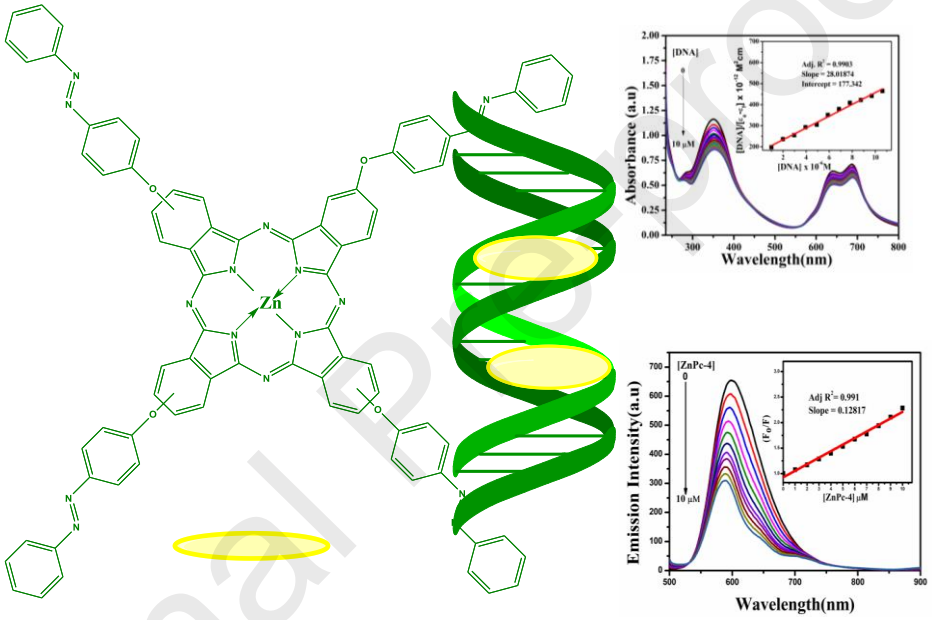

The DNA binding activity of the newly synthesized peripherally tetra-(4-phenylazophenoxy) substituted zinc phthalocyanine ZnPc-4 was evaluated by UV-visible absorption titrations, fluorescence quenching titration and viscosity measurements studies. The inferences from all the techniques invariably pointed out that ZnPc-4 intercalating into the base pair sequences of DNA by its large delocalized π surface. ZnPc-4 was also showing strong static interaction with BSA proven that compound is an effective DNA interacting drug structural candidate with biocompatibility.