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Synthesis of Diquinolineno[1,3,7,9]Tetraazacyclododecine-7, 15 (14*H*, 16H)-Dibenzene, and DNA Binding Studies of Macrocyclic Co(II), Cu(II) Complexes: As New Class of Antimicrobial Agent

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Synthesis of Diquinolineno[1,3,7,9]Tetraazacyclododecine-7, 15 (14*H*, 16H)-Dibenzene, and DNA Binding Studies of Macrocyclic Co(II), Cu(II) Complexes: As New Class of Antimicrobial Agent

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The synthesis of macrocyclic ligand, diquinolineno[1,3,7,9]tetraazacyclododecine-7, 15 (14H, 16H)-dibenzene(**L**), is described. The metal complexes of the type $[MLX_2]$, where (M = Co(II) (**1**), Cu(II) (**2**) and X = (Cl), have been synthesized by the reaction of ligand(**L**) with the corresponding metal salts, and characterized by elemental analysis, FT-IR, ^{1}H-NMR and electronic spectra. The binding property of the complexes with CT-DNA was studied by absorption spectra, viscosity measurements, as well as thermal denaturation studies. The absorption spectral results indicate that the complexes (**1**) and (**2**) are binds with base pairs of DNA. The intrinsic binding constant K_b had the value $3.8 \times 10^4 M^{-1}$ for (1) and 3.3×10^4 M^{-1} for (**2**), respectively, in 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2. The viscosity measurement results show the viscosity of sonicated rod-like DNA fragments increased when the complex were added to the solution of calf thymus-DNA. The synthesized ligand and its metal complexes have been screened for antibacterial and antifungal activities.

Keywords: Quinoline, macrocyclic complexes, DNA binding, viscosity measurements, antimicrobial activity

1. Introduction

The high selectivity and strong coordination ability of macrocyclic ligands towards transition metal ions have attracted attention of chemists all over the world due to the wide range of applications in areas like catalysis (1–3) electron carriers in redox reactions (4), dioxygen carriers (5, 6), ionospheres in a number of biochemical processes (7–9), separation and extraction of valuable and precious metals from waste materials (10), as anti-tumor drugs (11), as model compounds that mimic naturally occurring metalloproteines (12), and metalloenzymes used as photosensitizers in photodynamic therapy (PDT) (13). Thus, the transition metal complexes of mixed donor macrocyclic ligands constitute a potentially important class of molecules for molecular electronics and catalytic reductions (14).

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Macrocyclic complexes were best prepared with the aid of metal ions as template to direct the steric course of the condensation reaction, which ultimately results in ring closure (15). Various macrocyclic ligands have been synthesized and their complexes have been reported (16, 17). A variety of macrocyclic complexes derived from *o*-phenylinediamine including dinuclear macrocyclic complexes have been reported (18, 19).

The interaction of transition metal complexes with DNA has been extensively studied in the past few years. Among the first row transition metal ions, such as cobalt, nickel, manganese and copper offer the choice of biocompatibility in biological systems and have been recognized as having important biological effects (20). The study of DNA binding properties and anti-tumor activity of these metal complexes have been well documented in the literature (21, 22). Barton and co-workers (23, 24) have studied the interaction of enantiomers of $Ru(phen)_3$ with various DNA; the results lead them to the conclusion that there were two modes of interaction, intercalative and electrostatic binding. Kharatishvili et al. (25) also reported the effect on DNA binding in the presence of a planar intercalating ligand such as quinoline for both mononuclear and

dinuclear platinum (Pt) complexes. Recently, the investigation based on DNA interaction with macrocyclic complexes have great importance in understanding the action mechanism of some anti-tumor and anti-viral drugs, also to design new DNA targeted drugs and to screen these drugs *in vitro* (26).

The present paper discusses the synthesis of diquinolineno [1,3,7,9] tetraazacyclododecine-7,15 (14*H*, 16H)-dibenzene, DNA binding and antimicrobial studies of the title complexes.

2. Experimental

All chemicals used for the synthesis were of analytical grade and were procured from Sigma Chemical Co., U.S.A., E. Merck, Germany, Sarabhai Merck Company, India and *o*-phenylinediamene was purchased from S. D. Fine Chemicals Pvt. Ltd. The TLC was performed on Baker-Flex silica gel 1B-F (1.55) plates in the following solvent systems: ethyl acetate and petroleum ether (8:3). Melting points were determined on a Mel-Temp apparatus and are uncorrected. IR spectra were recorded in the matrix of KBr with a Perkin-Elmer 1430 spectrometer. ^{1}H NMR spectra were recorded on a Jeol spectrometer (400 MHz), and chemical shifts (δ) are given in ppm relative to the signal for TMS as internal standard. C, H and N analyses were performed at Cochin University, Sophisticated Test & Instrumentation Center, Kochi, Kerala, India. Conductivity measurements were determined in DMF (10^{-3} M) using an ELICO-CM82 Conductivity Bridge. Ultraviolet-visible absorption spectra were determined in a Perkin–Elmer model 554 with a Shimazdu UV-Vis recording spectrophotometer using quartz cuvettes of 10 mm light-path.

2.1. *Preparation of N,N′-bis[(2-chloroquinolin-3-yl) methylene] benzene-1,2-diamine(2a)*

The ethanolic solution of 2-chloro-3-formyl-quinoline (7.64 g, 0.04 mol) and *o*-phenylinediamine (2.16 g, 0.02 mol) (25 ml each) in 2:1 molar ratio was refluxed for 3-4 h. A yellowish product separates out and it was washed with cold ethanol, dried under vacuum, and recrystallized from ethyl acetate/dichloromethane solvent system. Yield 93%, m.p.120–122°C.

2.2. *Preparation of Diquinolineno[1,3,7,9] tetraazacyclododecine-7,15 (14H, 16H)-dibenzene (L)*

The compound **2a** (4.55 g, 0.01 mol) was dissolved in (25 ml) DMF and added to the (25 ml) *o*-phenylinediamine (1.28 g, 0.01 mol) in 1:1 molar ratio. The solution was refluxed in the presence of potassium carbonate (1.61 g, 0.01 mol) as catalyst for 10–12 h. The reaction was monitored by TLC using petroleum ether and ethyl acetate (8:3) as eluent. A greenish white precipitate was separated in ice cold water. The resulting product was collected by filtration, washed with cold water, dried under vacuum, and recrystallized from ethanol. Yield 85% m.p.185–187°C, FT-IR cm^{-1} 3430 (-NH-); 2924 (Ar-CH); 1619 (C=N); (other peaks) 1451, 1277, 1032, 924, 648. ^{1}H-NMR ($CDCl_3$)δ: 8.86 (s, 1H, NH); 7.6–8.0 (m, 8H, Ar-H, o-phenylinediamene); 8.93 (s, 1H, NH); 8.82 (d, 1H, CHN, D_2O exchangeable proton); 7.2–7.5 (m, 10H, Ar-H, quinoline)

2.3. *General procedure for the preparation of complexes*

A simple method has been adopted for the preparation of the complexes. The hot ethanol solution of ligand (**L**) and hydrated metal salt in 1:1 molar ratio were mixed. The mixture was refluxed for about 3–4 h, at 80 ± 5°C, the obtained residue was recrystallized from ethanol. Various attempts to develop the crystals suitable for X-ray diffraction studies such as slow diffusion, crystallization using mixtures of solvents and low temperature crystallization were unsuccessful.

2.4. *[Co(L)Cl$_2$]nH$_2$O:Cobalt(II) complex with ligand(L) diquinolineno[1,3,7,9] tetraazacyclododecine-7, 15 (14H, 16H)-dibenzene*

Ligand (**L**) was dissolved in (25 ml) ethanol and added to the hot ethanolic solution of cobalt(II) chloride (25 ml) in 1:1 molar ratio under boiling conditions and refluxed for 3–4 h. A blue colored precipitate formed was collected by filtration and dried. Similarly, the same procedure was followed for Cu(II) complex, and the experimental data were summarized in the Table 1.

2.5. *DNA binding experiments*

The concentration of CT-DNA per nucleotide [C(p)] was measured by using its known extinction coefficient at 260 nm (6600 M^{-1}cm^{-1}) (27). The absorbance at 260 nm (A_{260}) and at 280 nm (A_{280}) for CT-DNA was measured to check the purity. The ratio A_{260}/A_{280} was found to be 1.8–1.9, indicating that CT-DNA was adequately free from protein. Buffer [5 mM tris(hydroxymethyl)aminomethane, pH 7.2, 50 mM NaCl] was used for the absorption, viscosity, and thermal denaturation experiments.

Absorption titration experiments were carried out by varying the DNA concentration (0–100 μM) and maintaining the metal-complex concentration constant (30 μM). Absorption spectra were recorded after each successive addition of DNA and equilibration (approximately 10 min). For both complexes (**1**) and (**2**), the observed data were then fit in to (1) to obtain the intrinsic binding constant, K_b (28):

$$[\mathrm{DNA}] / (\varepsilon_a - \varepsilon_f) = [\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f) + 1/\mathrm{K_b}\,(\varepsilon_b - \varepsilon_f) \quad (1)$$

Table 1. Analytical and physical properties of the metal complexes diquinolineno[1,3,7,9] tetraazacyclododecine-7,15 (14H, 16H)-dibenzene(L)

Complex	*Color*	*Molecular Wt (Yield %)*	*m.p.°C*	*Meff (B.M)*	*($\Delta m\ \Omega^1$ cm^2mol^{-1})*	*Elemental analysis Calcd. (Found %)*
(L) $C_{32}H_{22}N_6$	Greenish white	490.55 (85)	180	—-	—-	C:78.35 (78.26) H:4.52 (4.58) N:17.13 (17.05)
$[Co(L)Cl_2]$ (1) $C_{32}H_{22}N_6\ Cl_2Co$	Dark bluish	549.49 (83)	>250	2.56	72	C:69.95 (69.88) H:4.04 (4.06) N:15.29 (15.31) Co:10.7 (10.0)
$[Cu(L)Cl_2]$(2) $C_{32}H_{22}N_6Cl_2Cu$	Reddish	554.10 (71)	>280	1.99	75	C:69.36 (69.28) H:4.00 (3.97) N:15.17 (15.19) Cu:11.47 (11.39)

Where $\varepsilon_{a,}\varepsilon_{f,}$ and ε_b are the apparent, free, and bound metal complex extinction coefficients at 304 nm, 344 nm for Co(II), 302 nm, 347 nm for Cu(II), respectively. A plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs. [DNA] gave a slope of $1/(\varepsilon_a - \varepsilon_f)$ and a y intercept equal to $1/K_b(\varepsilon_a - \varepsilon_f)$, where K_b is the ratio of the slope to the y intercept. Viscosity measurements were carried out using a semi-micro dilution capillary viscometer at room temperature. Each experiment was performed three times and an average flow time was calculated. Data were presented as (η/η_o) vs. binding ratio, where η is the viscosity of DNA in the presence of complex and η_o is the viscosity of DNA alone. Thermal denaturation experiments were carried out by monitoring the absorption of CT-DNA (50 μM) at 260 nm at various temperatures in the presence (5–10 μM) and the absence of each complex. The melting temperature [(T_m), the temperature at which 50% of double-stranded DNA becomes single-stranded] and the curve width σT, the temperature range between which 10% and 90% of the absorption increases occurred were calculated as reported elsewhere (29, 30).

3. Results and discussion

A novel diquinolineno[1,3,7,9] tetraazacyclododecine-7,15 (14*H*, 16H)-dibenzene, macrocyclic ligand (**L**) has been synthesized in two steps as per Scheme 1. In the first step the *o*-phenylinediamine reacts with 2-chloro-3-formyl-quinoline, in 1:2 molar ratio in ethanol, a yellowish colored product N-[-(2-chloroquinolin-3-yl) methylene]-*N*-(2-chloroquinolin-3-yl) methylene] benzene-1,2-diamine separated out, in a second step, it reacts with *o*-phenylinediamine in 1:1 molar ratio in DMF solvent, gave a greenish white colored solid. The TLC has established the purity of the compound by dissolving the ligand in ethanol using petroleum ether and ethyl acetate (8:3) as eluent. One spot was observed in the TLC plate after developing in an iodine chamber indicating that the compounds were pure. The formation of this macrocyclic molecule framework was confirmed based on the results of FT-IR and resonance peaks in the ^{1}H-NMR and elemental analyses. By using this, new macrocyclic complexes of the type $[MLX_2]$, were synthesized by the reaction of the ligand(**L**) with the corresponding metal salts in 1:1 molar ratio in ethanol solution (Figure 1).

The formation of the complex may be represented by the following reaction:

$$CoX_2.H_2O + L \rightarrow Co(L)X_2 + nH_2O$$
$$CuX_2.H_2O + L \rightarrow Cu(L)X_2 + nH_2O$$

The complexes are microcrystalline in nature and found to be soluble in most of the organic solvents. The elemental analysis data shows that the complexes have a composition of $[Co(L)Cl_2]$, $[Cu(L)Cl_2]$. The magnetic moment value 2.56 for Co(II), and 1.99 for Cu(II), which are greater than spin-only value 1.75 (B.M) and hence, paramagnetic in nature, exhibits high–spin octahedral geometry. The coordination spheres of complexes, similar to those of Nickel(II)-type macrocyclic complexes, have been reported to be six-coordinate octahedral geometry (12). Hence, in the present studies, the experimental results suggest that the title complexes possess octahedral geometry. Molar conductivity was studied in DMF, the range of 72–75 Ω^{-1} cm^{-1} mol^{-1} indicating that both the complexes are 1:1 electrolytes and may be formulated as $[MLX_2]$.

3.1. *FT-IR spectra*

IR spectra of complexes were recorded in the matrix of KBr pellets with a Perkin-Elmer 1430 spectrometer. The absence of bands corresponding to the amino groups of o-phenylinediamene and carbonyl groups of aldehydic 2-chloro-3-formyl-quinoline, suggests the formation of the proposed macrocyclic ligand (**L**). Further, the two intensive bands at 1619 cm^{-1} and 3430 cm^{-1} assignable to uncoordinated ν(C=N) and ν(N−H) of amine group, respectively

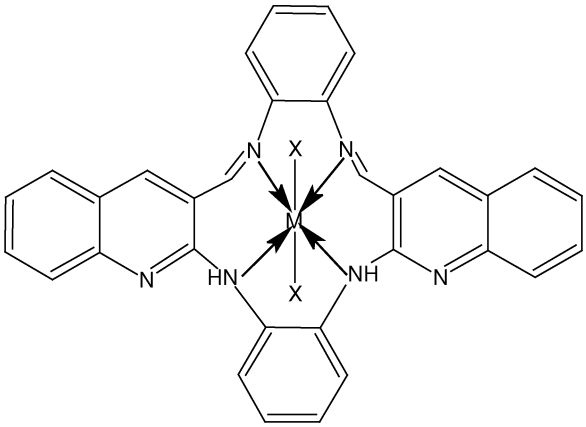
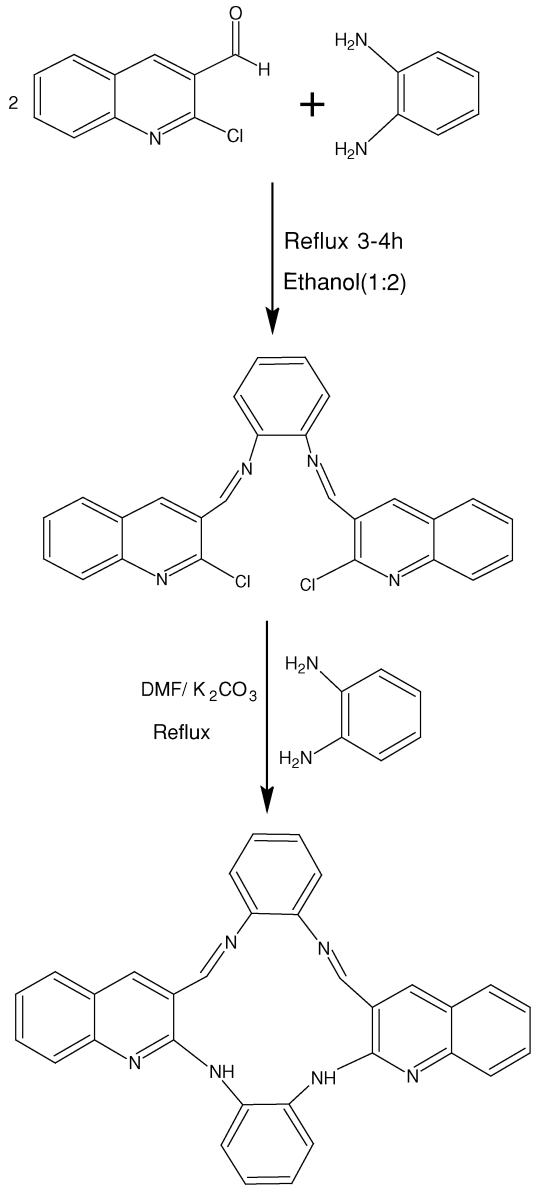


Fig. 1. Suggested structure of $[M(L)X_2]$ where (M= Co(II), Cu(II) X=Cl).

confirms the proposed structure (31, 32). In addition, the formation of macrocyclic structure was conformed by its ^{1}H-NMR spectra. However, IR spectra of complexes derived from the ligand (**L**) shows a slight shift to the lower frequency in υ(C=N) and appeared in the region 1594–1607 cm^{-1} suggesting its coordination with metal ion. In addition, a strong characteristic band of υ(-NH-) appeared at 3176 cm^{-1}, and bands at 1451–1415 cm^{-1} for all the complexes correspond to C−H binding vibrations, respectively. The appearance of new medium–intensity bands in the region 761–768 cm^{-1} in the macrocyclic complexes may be assigned to υ(M-N) vibrations. The bands at 451–479 cm^{-1} were assigned to υ(M-Cl) vibrations, and the values are summarized in Table 2.

3.2. *^{1}H-NMR spectra*

The ^{1}H-NMR spectra were recorded on a Jeol spectrometer (400 MHz), and chemical shifts (δ) are given in ppm relative to the signal for TMS as the internal standard. The absence of proton resonance signals of free NH_2 and aldehydic (CHO) groups indicates the condensation between amine and carbonyl group of aldehydic 2-chloro-3-formylquinoline. The ^{1}H-NMR spectra of the ligand recorded in $CDCl_3$ show a doublet at δ: 8.82 ppm (d, 1H, CHN D_2O exchangeable), may be due to hydrogen bonding and anisotropy effect of the adjacent and other aromatic resonated protons, and signal exhibits singlate at δ: 8.86 ppm (s, 1H, NH) and 8.93 (s, 1H, NH) ascribed. A multiplet signal at 8.2–8.56 ppm (m, 8H, Ar-H,) corresponds to the aromatic o-phenylinediamine. The multiplet signals attributed at 7.0-7.56 ppm are due to (m, 10H, Ar-H,) aromatic quinoline moiety.



Sch. 1. Synthesis of diquinolineno[1,3,7,9] tetraazacyclododecine-7,15 (14*H*, 16H)-dibenzene

Table 2. Characterizations of IR cm^{-1} bands of ligands and their metal complexes

Compound	*υ(N−H)*	*υ(Ar−CH)*	*υ(C=N)*	*υ(C=C)*	*υ(C−H)*	*υ(M−N)*	*υ(M−Cl)*
(L) $C_{32}H_{22}N_6$	3430 s	2924 m	1619 s	1488 m	1451 s	—	—-
$[Co(L)Cl_2]$ (1) $C_{32}H_{22}N_6Cl_2Co$	3186 s	2854 m	1607 s	1458 m	1416 s	761 s	479 m
$[Cu(L)Cl_2]$(2) $C_{32}H_{22}N_6Cl_2Cu$	3177 s	2851 m	1594 s	1459 m	1415 s	768 s	451 m

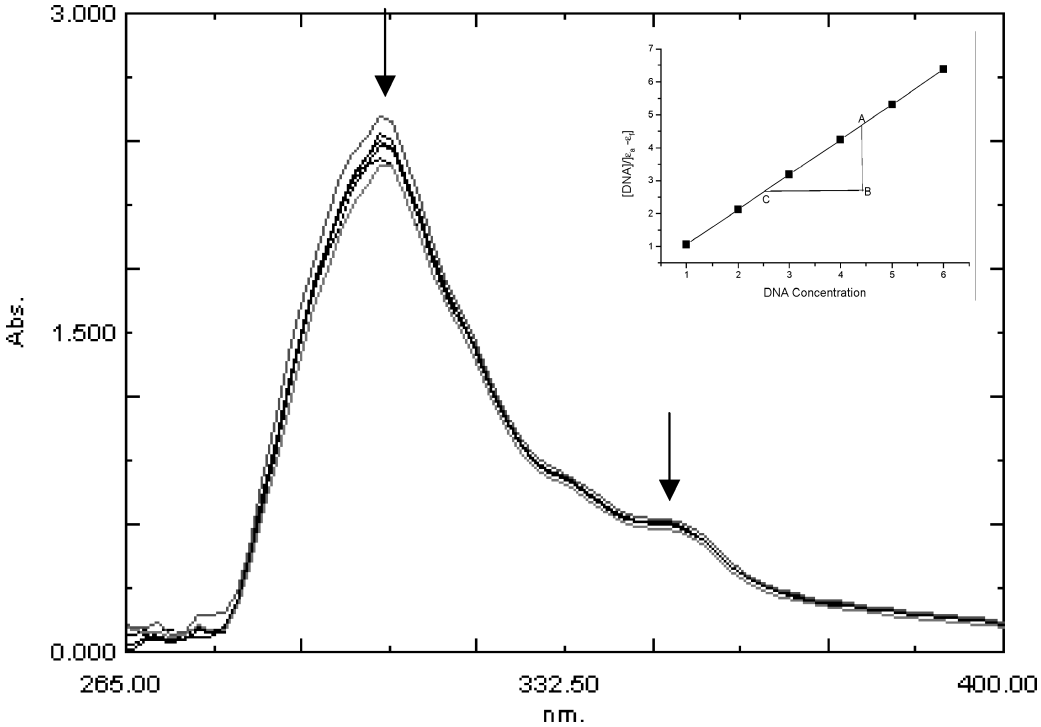


Fig. 2. Absorption spectral traces of complexes $[Co(L)Cl_2]$ (1) in *Tris*-HCl buffer (0.01 M, pH 7.2) upon addition of CT-DNA = 0.5 μm, = 10 μm, drug, 20 μm; 30 μm; 40 μm; 50 μm; arrow shows the absorbance changing upon increase of DNA concentration.

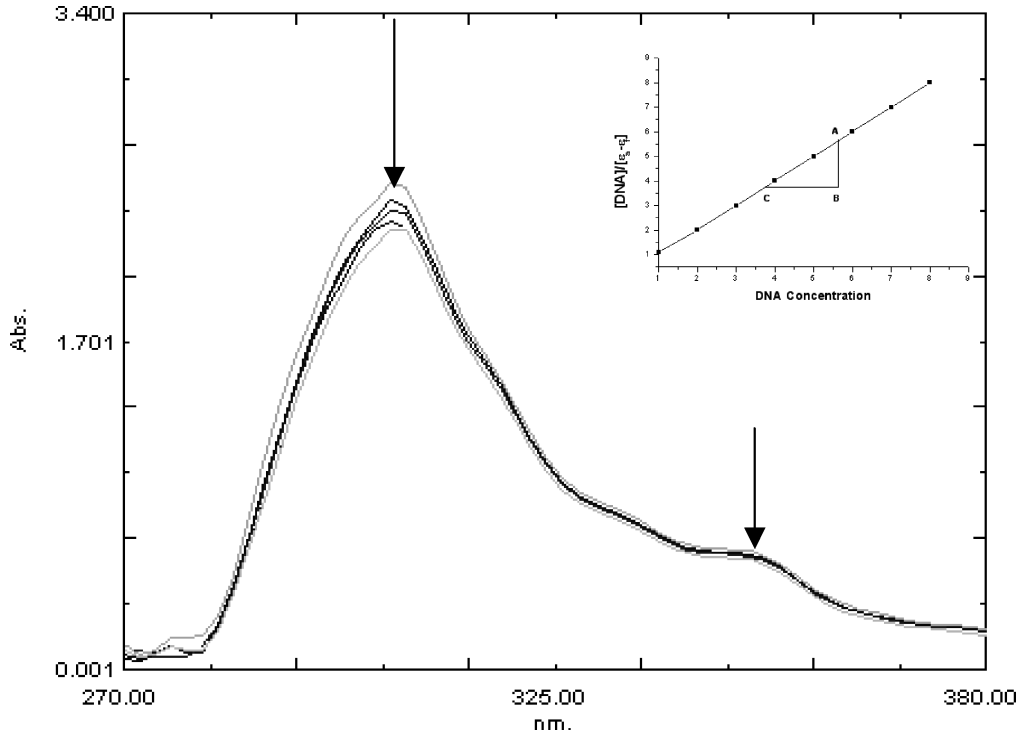


Fig. 3. Absorption spectral traces of complexes $[Cu(L)Cl_2]$ (2) in *Tris*-HCl buffer (0.01M, pH 7.2) upon addition of CT-DNA = 0.5 μm, = 10 μm, drug, 20 μm; 30 μm; 40 μm; 50 μm; arrow shows the absorbance changing upon increase of DNA concentration.

3.3. *Absorption spectral features of DNA binding*

Electronic absorption spectroscopy is universally employed to determine the binding of complexes with calf thymus-DNA (20). The absorption spectra of complex (**1**), and (**2**) shows a well resolved absorption bond at 304 nm, 344 nm for (**1**) and 302 nm 347 (**2**), respectively. In the presence of increasing amounts of CT-DNA, both complexes (**1**) and (**2**) showed a strong decrease in intensity (hypochromicity: 8% for Co(II) and 6% for Cu(II) complexes) and bathochromic shifts (maximum: 3 ± 1 nm for cobalt(II) and 2 ± 1 nm for copper (II) complexes) for their most red-shift absorption peak maxima (Table 3). The change in the absorbance values (at 304 nm and 344 nm for complex (**1**) and at 302 nm and 347 nm for complex (**2**)) with increasing amounts of CT-DNA were used to evaluate the intrinsic binding constants (K_b) for the complexes (Figures 2 and 3). The values of K_b evaluated for ligand, complexes (**1**) and (**2**), using Equation 1, 2.8×10^3 M^{-1} for ligand, $3.8 \times 10^4 M^{-1}$ for (**1**) and 3.3×10^4 M^{-1} for (**2**), respectively. This value suggested that the complexes are bound more avidly to CT-DNA than the ligand. The observed K_b values are comparable to those observed for typical classical intercalators [EthBr, K_b, $1.8 \times 10^6 M^{-1}$ in 25 mM Tris-HCl/40 mM NaCl buffer, pH 7.9) and partial intercalating metal complexes [Ru(phen)2(dppz)]2+, dppz = dipyrido-[3,2-d: 2′,3′-f]-phenazine, $K_b > 10^6 M^{-1}$] bound to CT-DNA (33, 34).

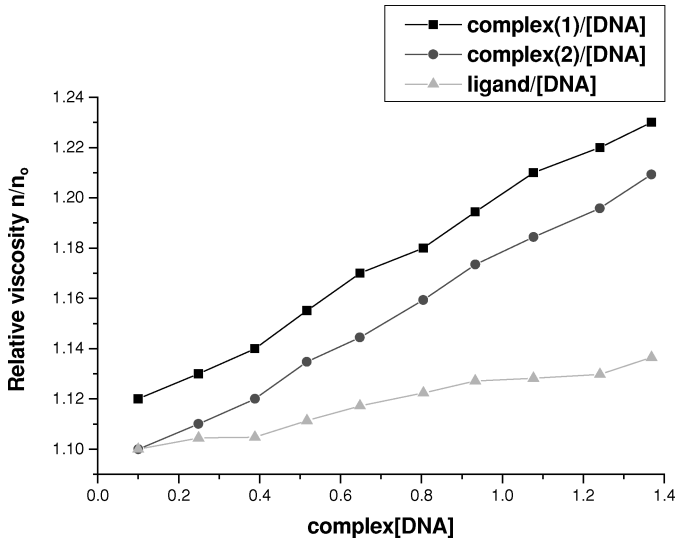


Fig. 4. Effects of increasing amount of ligand and Complex (**1**), (**2**) on the relative viscosity of CT-DNA at 25 ± 0.1°C.

3.4. *Viscosity measurements*

The viscosity measurements further clarified the binding modes of complexes with CT-DNA. Hydrodynamic measurements that are sensitive to length change (for examples, viscosity, sedimentation) are regarded as the most critical tests of binding in solution in the absence of crystallographic structure data (35). A classical intercalative mode causes a significant increase in viscosity of DNA solution due to an increase in separation of base pairs at intercalation sites and hence, an increase in overall DNA length. By contrast, complexes that bind exclusively in the DNA grooves by partial and/nonclassical intercalation, under the same conditions, typically cause negative or no change in DNA solution viscosity (36, 37). In order to further elucidate the binding mode of the present complex, the viscosity measurements were carried out on CT-DNA by varying the concentration of added complex. The effects of the complex (**1**) and (**2**) on the viscosity of rod-like DNA were shown Figure 4. As expected for the complex (**1**) and (**2**), the viscosity of DNA increases with an increase in concentration of the added complex. The results revealed that the presence of the complex has an obvious effect on relative viscosity of CT-DNA.

Table 3. Absorption spectral properties macrocyclic ligand and complexes of Co(II) and Cu(II) bound to calf thymus-DNA

Complex	λ_{max} *(nm)*	$K_b(M^{-1})$	T_m(°C)
Ligand/[DNA]	355	2.8×10^3	54
Complex (1)	304	3.8×10^4	63
Complex (2)	302	3.3×10^4	65

3.5. *Thermal denaturaration studies*

Additional information on the DNA binding properties was obtained from melting studies. The stability of the DNA helix with temperature indicates an interaction between DNA to metal complex in the concentration ratio of 25 and (T_m) values were determined by monitoring the absorbance of DNA at 260 nm as a function of temperature. In the present study, when the complex solutions are added to the DNA solution, the melting temperature is increased. This indicates that there is an interaction between DNA and metal complexes. The melting of DNA in the absence of any complex was found to be 60 ± 1°C, under the same experimental conditions, the presence of complexes (**1**) and (**2**) increased the melting temperature by about 3 to 6°C, as shown in Figure 5. Likewise, they stabilized the double strand of calf thymus-DNA (38, 39).

3.6. *Evaluation of antimicrobial activity*

The *in vitro* antimicrobial activity was carried out against 24 h old cultures of two bacteria and two fungi by a cup-plate method (40). Complexes have been tested for their antibacterial activity against *Pseudomonas aerugenosa* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger* and *Candida albicans.* Nutrient agar and potatodextrose agars were used to culture the bacteria

Table 4. Antimicrobial activity of ligand and its complexes.

	Antibacterial activity Zone of inhibition in mm		*Antifungal activity Zone of inhibition in mm*	
Complex	*P. aerugenosa*	*S. Aureus*	*A. niger*	*C. albicans*
(L) $C_{32}H_{22}N_6$	19	20	21	20
$[Co(L)Cl_2](1)$ $C_{32}H_{22}N_6Cl_2Co$	17	19	18	17
$[Cu(L)Cl_2](2)$ $C_{32}H_{22}N_6Cl_2Cu$	17	18	19	18
Chloramphenicol	22	24	—	—
Flucanazole	—	—	25	26

and fungus, respectively. The compounds were tested at a concentration of 0.005 mol/ml in a DMSO solution. The solution of Chloramphenicol (2 mg/ml) and Flucanazole (2 mg/ml) were prepared in sterilized water and used as standards for comparison of antibacterial and antifungal activities, respectively. The compounds were tested at varied concentration. The minimum inhibition concentration was found to be 0.001 mol/ml in DMSO against all organisms. Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria at 28°C and 48 h for fungus at 35°C. Each experiment was repeated three times and the average of the three independent determinations was recorded. The protocols were summarized in Table 4. The result of the inhibitory activity of the ligand and its complexes on a few species of bacteria and fungi are given in the Table 4. The data shows that the ligand exhibited significant inhibitory activity compared to its metal complexes.

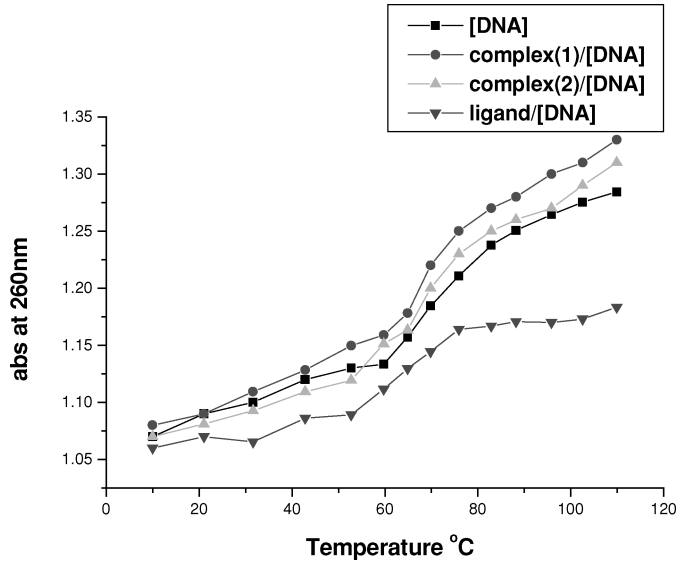


Fig. 5. Melting curves of CT-DNA in the presence and absence of ligand and complex **(1)** and **(2).**

4. Conclusions

The synthetic route adopted for synthesis of macrocyclic ligand and its metal complexes of the type $[M(L)X_2]$ was very simple and gave good yield. In DNA binding studies, the absorption spectral results indicate hypochromicity and bathochromic shifts (red shift) of the complex **(1)** and **(2)** when it binds with base pairs of calf thymus-DNA. The binding constant values of ligand (2.8×10^3 M^{-1}), and complexes (3.8×10^4 M^{-1}) for **(1)** and **(2)** (3.3×10^4 M^{-1}) suggested that the complexes bind more avidly to CT-DNA than the ligand. In addition, increasing the viscosity of sonicated rod-like DNA fragments and the melting temperature of DNA, in the presence of complex solutions supports the binding mode. The antimicrobial activity shows both the ligand and its complex have exhibited significant inhibitory activity.

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