Synthesis, Characterization, Electrochemical and DNA Interaction Studies of New Macrocyclic Binuclear Cu(II) Complexes

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Abstract— **The design and synthesis of metal-containing macrocycle is an interesting field . A new series of binuclear copper(II) complexes Cu_2L^{1-6} were synthesized by using template method. The mononuclear complexes CuL^{1-6} were prepared by the condensation of alkyl diamines, aryl diamines with 2,6-diformyl-4-methylphenol and Copper acetate dihydrate in the ratio of 1:2:1 in acetonitrile. The above mononuclear complexes were condensed insitu with a mole of Copper perchlorate hexahydrate in the presence of one mole of 1,3-diamino Guanidine hydrochloride. The resulting macrocyclic binuclear complexes Cu_2L^{1-6} were characterized by elemental analysis, spectral and electrochemical studies. The redox behaviour was investigated by cyclic voltammetry and it showed metal centered reduction process for all complexes. The reduction and oxidation potential depends on the structure and conformation of the central atom in the coordination compounds. The complexes showed first reduction potential in the range of (E^1_{Pc} = -0.52 to -0.96 V) and the second reduction potential in the range of (E^2_{Pc} = -1.02 to -1.39 V) versus Ag/AgCl in DMF and the ΔE values were found to be greater than 60 mV, indicating that the nature of the single electron transfer processes were quasi-reversible. The interaction of the complexes with calf thymus DNA were studied using absorption and fluorescence spectroscopic techniques.**

I. INTRODUCTION

Cisplatin is a widely used and well-known drug for cancer therapy,[1] which inhibits the proliferation of cancer cells through DNA- or mitochondria-based paths.

DNA is an important target for anticancer drugs because it plays a central role in the replication, transcription, and regulation of genes. Some new non-platinum anticancer metallodrugs can bind to DNA by intercalation.[2] Metal complexes with extended aromatic ligands can bind to DNA in a non-covalent fashion, especially through intercalative or partial intercalative binding.[3] Considerable studies of these complexes need to be done in order to develop the complexes binding to DNA through an intercalation mode, with ligands achieving fully planar intercalation into the adjacent base pairs of DNA.[4] Due to the key role of DNA in cell life, the design and synthesis of metal-based. The clinical success of cisplatin and related platinum-based drugs, as anticancer agents that bind covalently to DNA, is severely affected by the serious side effects, general toxicity, and acquired drug resistance.[5-8] This is an impetus to inorganic chemists to develop innovative strategies for the preparation of more effective, less toxic, target specific, and preferably noncovalently bound anticancer drugs. Many studies suggest that DNA is the primary intracellular target of antitumor drugs, because the interaction between small molecules and DNA can cause DNA damage in cancer cells.[9–11] Recently, Ru(II), Rh(II), V(IV), Fe(III), Co(III), Ni(II) and Cu(II),[12-19] were widely explored as sources of metal ions used in the DNA binding.

Many metal complexes have been synthesized using various modified ligands with the purpose of enhancing their interaction with DNA [20,21] The guanidine group defines chemical and physiochemical properties of many compounds of medical interest and guanidine-containing derivatives constitute a very important class of therapeutic agents suitable for the treatment of a wide spectrum of diseases. Recent achievements in the synthesis of guanidine-containing molecules with diverse chemical, biochemical and pharmacological properties make them of great importance to the design and development of novel drugs acting at CNS, anti-inflammatory agents, antithrombotic, antidiabetic and mainly as a chemotherapeutic agents as well as guanidinium-based transporters and vectors. However, most of the studies have mainly focused on metal complexes containing fully planar ligands, while metal complexes containing substituted ligands have been rarely reported. In fact some of these complexes also exhibit very interesting properties upon binding to DNA [22–24].

A variation in the nature and position of the substituents at the binding site of the ligand can create some interesting differences in the space configuration and electron density distribution of the metal complexes,

resulting in differences in spectral profiles, and DNA-binding properties.Herein, we present the synthesis, structure, DNA binding activity of new the macrocyclic Cu(II) complexes.

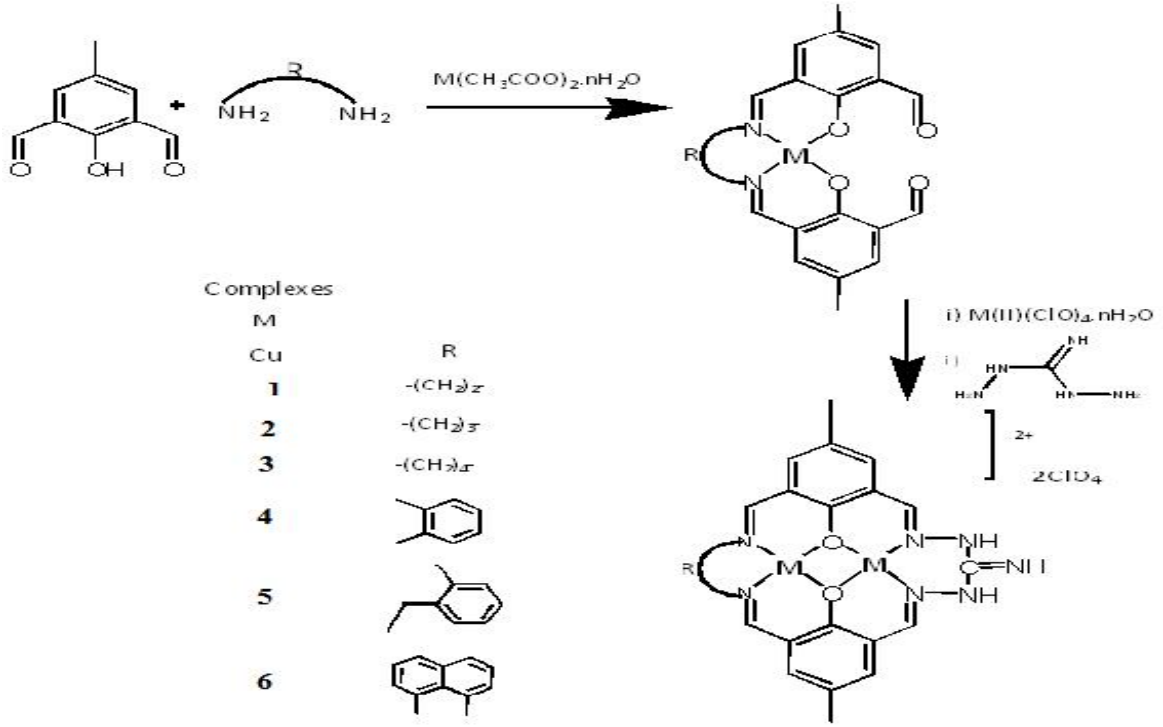
II. EXPERIMENTAL SECTION

1.1Materials and measurements:

2,6-diformyl 4-methyl phenol and the macrocyclic binucleating ligands L^1 to L^6 were prepared using the procedure provided in the literature[25,26] with slight modifications. Tetra butyl ammonium perchlorate (TBAP) was purchased from Fluka and recrystallized using hot methanol. (Caution! TBAP is potentially explosive; hence, care should be taken when handling the compound.) Sodium salt of 4-nitrophenyl phosphate (4-NPP) was purchased from Aldrich. CT DNA and pBR322DNA were purchased from Bangalore Genie (India). All other chemicals and solvents were of analytical grade and used as received, without any further purification. FT-IR spectra were obtained on a Perkin Elmer FTIR spectrometer with samples prepared as KBr pellets. UV-visible spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer operating in the range of 200–1100 nm with quartz cells and e are given in $M^{-1}cm^{-1}$. Cyclic voltammetric measurements were made at 25 °C on a CH11008 Electrochemical analyzer using a three-electrode setup comprised of glassy carbon working, platinum wire auxiliary and Ag/Ag+ reference electrodes under oxygen free conditions. The concentration of the complexes was 10^{-3} M. TBAP (1021 M) was used as the supporting electrolyte. Concentrated stock solutions of DNA (10^{-5} mM) were prepared in a buffer. The concentration of DNA determined by UV absorbance at 260 nm after 1 : 100 dilutions. The extinction coefficient, $\sum_{260}$, was taken as 6600 $M^{-1}cm^{-1}$. Stock solutions were stored at 4 °C.

1.2.Synthesis of binucleating ligand L^1 :

The ligand L^1 was prepared by the Schiff base condensation of two moles 2,6 diformyl-4-methyl phenol with one mole of ethylene diamine. The pre-cursor compound 2,6 diformyl-4-methyl phenol(1.64g, 0.01 moles) taken in a 250 ml round-bottomed flask was dissolved in a mixture of $CHCl_3$(10ml) and CH_3CN(20ml) solvents. To the above solution the ethanolic solution of ethylene diamine was added dropwise with constant stirring. The reaction mixture was stirred for about 5 hours and the resulting yellow compound was filtered and dried. Similarly the ligands *$L^2$to L^6 were prepared*



SCHEME

1.3.Synthesis of binuclear copper complexes $[Cu_2L^{1to6}(ClO_4)_2]$;

The binuclear Cu(II) complexes 1 to 6 were prepared from a general synthetic procedure in which 1g(0.5 mmol) quantity of Cu(II) acetate monohydrate in 10 mL of methanol was added at 25° C to the Schiff base ligand $L^{1\text{ to }6}$ (0.5 mmol) taken in 20 mL of methanol and chloroform (2:1) mixture. The reaction mixture

was stirred for 15 minutes and kept for refluxing for about 20 minutes. To this mononuclear complex formed, an ethanolic solution of Cu $(ClO_4)_2 6H_2O$(1.85g, 0.5 mmol) was added dropwise to obtain a clear solution. To the above solution was added an aqueous solution of 1,3 diamino Guanidine Hydrochloride 0.62 g (0.5 mmol). The reaction mixture was refluxed for 8 hours and the resulting solution was filtered and dried to obtain the brown coloured binuclear Cu(II) complex.

Yield obtained for Cu_2L^1 0.12 g (77%); Pale yellow solid; IR (KBr, cm−1): broad band at 3450 ν(OH), 1635 ν(C N), 1110 ν($ClO4^-$), 611 ν($ClO4^-$). UV absorbance at λmax 248 (185,000), nm (ε, M^{-1} cm^{-1}) in DMF: CT at 310 (18900). Elem.Anal: Calcd for Cu_2L^1,$C_{23}H_{25}Cu_2N_7O_2$; Cal for C,49.45; H,4.51; Cu,22.75; N,17.55; O,5.75; Found; C,49.42; H,4.49; Cu,22.71; N,17.53; O,5.72;

1.4. DNA binding experiments:

1.4.1 Absorption spectral studies:

The DNA binding experiments were performed in Tris-HCl/NaCl buffer (50 mM Tris HCl/ 1 mM NaCl buffer, pH 7.5) using a dimethyl formamide (DMF) (10%) solution of complexes 1–6. The concentration of CT DNA was determined from the absorption intensity at 260 nm with a ε value of 6600 M−1 cm−1. Absorption titration experiments were made using different concentrations of CT-DNA, keeping the complex concentration constant.

1.4.2. Fluorescence spectral studies.

The fluorescence spectral method was used to determine the relative DNA binding properties of the dicopper(II) complexes 1–6 to CT DNA in 50 mM Tris- HCl/1 mM NaCl buffer, pH 7.5, using ethidium bromide (EB) as a reference. The fluorescence intensities of EB at 610 nm with an excitation wavelength of 510 nm were measured at different complex concentrations. Reduction in the emission intensity was observed with addition of the complexes.

III.RESULTS AND DISCUSSION:

3.1.General Properties :

A very strong and broad band near 1100 cm^{-1} is observed in the IR spectra of all the heterocyclic base coordinated complexes which is in agreement with the presence of uncoordinated ionic perchlorate in the complex lattices. The electronic spectra of the CH_3CN solution of all complexes showed only one intense intraligand band in the UV region. In the visible region, complexes exhibited absorption maxima in the range of 410 nm to 585 nm, respectively.

3.2.Electrochemical Studies:

The cyclic voltammogram of binuclear Cu(II) complexes 1–6 were measured in DMF from the potential region (-0.4 to -1.6 V) and the electrochemical data are summarized in Table 1. The redox behaviour was investigated by cyclic voltammetry and it showed metal centered reduction process for all complexes. The complexes showed first reduction potential in the range of (E^1p_c= -0.52 to -0.96 V) and the second reduction potential in the range of (E^2pc = -1.02 to -1.39 V) versus Ag/AgCl in DMF and the ΔE values were found to greater than 60 mV indicating that the single electron transfer processes were quasi-reversible. The cyclic voltammogram of Cu_2L^2 is shown in fig 1:

Fig. 1 Cyclic Voltammogram of binuclear Cu_2L^2 complex in the cathodic region [complex] = 1 x 10^{-3} M, [TBAP] = 1x 10^{-1}M.

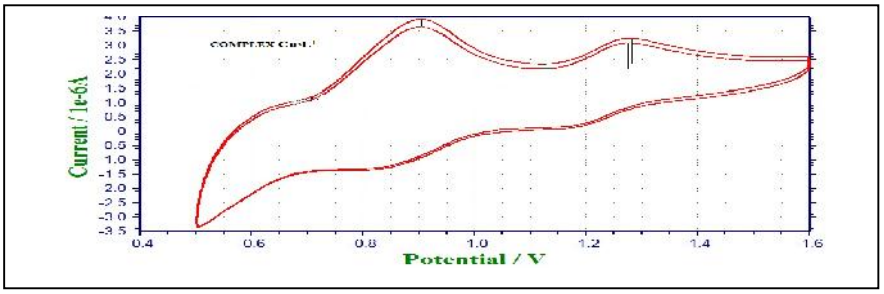


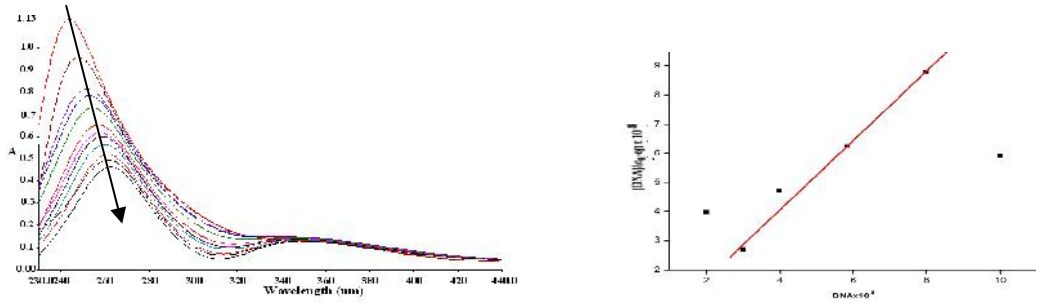
TABLE 1:Electrochemical data of the binuclear copper (1-6) complexes has been listed below :

3.3. DNA Binding Studies:

3.3.1. Absorption Spectral Studies:

Absorption titration technique has been used to monitor the mode of interaction of 1−6 with CT DNA. The naphthalene diamine containing complex 6 show higher K_b values in comparison to their other complexes possibly due to the coplanarity of the naphthalene system in the macrocyclic ring. Also, the complex 1 also showed better DNA binding propensity than the other aliphatic diamine containing complexes 2 and 3. The Kb values of~10^5 M^{-1} follow the order: 6 > 5 > 4 >1 > 2 > 3. The K_b value of Cu_2L^6 (complex 6) was calculated to be 4.56 x 10^5 M^{-1}.

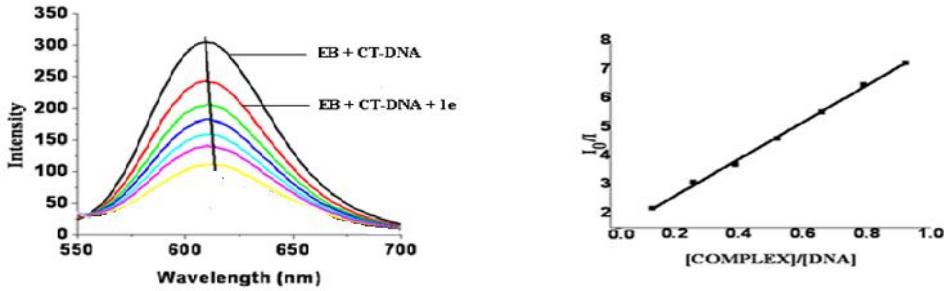
Fig 2 : Absorption spectra of dicopper (II) complex 6 in the absence and presence of increasing amounts of CT DNA (0–2.5 µM) at 25 ºC in 50 mM Tris-HCl/NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations.



3.3.2. Fluorescence Spectral Studies:

We have used fluorescence spectral titration method to obtain the apparent binding constant values (Kapp) of complexes 1–6 (Table 2). Ethidium bromide (EB) has been used as a spectral probe as it exhibits an enhanced emission intensity when it binds to the DNA. The competitive binding of the complexes to DNA could result in the displacement of bound EB and could cause a decrease in the emission intensity because of solvent quenching. The emission spectra of EB bound to DNA in the absence and presence of complex 6 is shown in Fig. 3. The K_{app} Value and the K_{sv} value of complex 6; Cu_2L^6 was calculated to be 3.2 x 10^6 M^{-1} and 3.3 respectively.

Fig. 3. The emission spectra of EB bound to DNA in the absence and presence of complex 6 is as shown below:



Complex	E^1p_c	E^1p_a	$E^1_{1/2}$	$(\Delta E^1 p)$	E^2p_c	E^2p_a	$E^2_{1/2}$	$(\Delta E^2 p)$
1	-0.97	-0.76	-0.86	210	-1.35	-1.27	-1.31	80
2	-0.89	-0.82	-0.855	70	-1.27	-1.19	-1.23	80
3	-0.75	-0.69	-0.715	60	-1.34	-1.24	-1.29	100
4	-0.77	-0.69	-0.725	80	-1.09	-1.01	-1.05	80
5	-0.94	-0.83	-0.885	110	-1.14	-1.04	-1.09	100
6	-0.98	-0.86	-0.92	120	-1.31	-1.22	-1.265	90

IV.CONCLUSION:

The Cu(II) Schiff base complexes were synthesized and characterized by electrochemical and spectroscopic techniques. The binding properties of the complexes have been investigated by using absorption and fluorescence spectral studies. Complexes 1–6 bind with the CT-DNA through intercalation with binding constants in the order of 10^5. The binding studies indicate that the binding propensity of complex 6 is dominant when compared to that with the other complexes 1-5.

REFERENCES:

[1].(a) C. Liu, M. Wang, T. Zhang and H. Sun, Coord. "DNA hydrolysis promoted by bi and multinuclear complexes" Chem. Rev., 2004, 248, 147;

[2] M. Klajner, P. Hebraud, C. Sirlin, C. Gaiddon and S. Harlepp. "DNA binding to an anticancer Organo-Ruthenium complex" J. Phys. Chem. B, 2010, 114, 14041; (b) P. Wang and N. Tang, J. Fluor- esc., 2009, 19, 1073;

[3] L. H. Hurley, Nat. Rev. " DNA binding properties and cytotoxicity of Cu(II) complex of Xanthone" Cancer, 2002, 2, 188.

[4] L. S. Lerman, J. "Structural consideration and the interaction of DNA" Mol. Biol., 1961, 3, 18.

[5] B. Rosenberg, L. VamCamp, J. E. Trosko and V. H. Mansour, "New class of potent Antitumour agents" Nature, 1969, 222, 385.

[6] E. R. Jamieson and S. J. Lippard, "Processing of Cisplatin-DNA adducts" Chem. Rev., 1999, 99, 2467.

[7] F. Arnesano and G. Natile, Coord. "Mechanistic insight into the cellular uptake and processing of Cisplatin"Chem. Rev., 2009, 253, 2070.

[8] A. V. Klein and T. W. Hambley, "Advances in Anticancer agents in medicinal chemistry" Chem. Rev., 2009, 109, 4911.

[9] K. S. Soppimath, L. H. Liu, W. Y. Seow, S. Q. Liu, R. Powell, P. Chan and Y. Y. Yang, "Adv. Funct. Mater.", 2007, 17, 355.

[10] A. Nori and J Kopecek, "Adv. Drug Delivery" Rev., 2005, 57, 609.

[11].Y.Xie,G.G.Miller, S.A.Cubitt, K.J.Soderlind, M.J.Allalunis-Turner and J. W. Lown, "Anti-Cancer Drug Des' "Antisense Therapy", 1997, 12, 169.

[12]. M. H. Lim, H. Song, E. D. Olmon, E. E. Dervan and J. K. Barton, "Sensitivity of Ru-complexes and luminescence to DNA defects'' Inorg. Chem., 2009, 48, 5392.

[13] Y. J. Sun, L. E. Joyce, N. M. Dickson and C. Turro, "Efficient Photocleavage by Ru(bpy)$_2$complexes with visible light''. Chem. Commun., 2010, 46, 2426.

[14]M. H. Lim, H. Song, E. D. Olmon, E. E. Dervan and J. K. Barton, "Sensitivity of Ru-complexes and luminescence to DNA defects'' Inorg. Chem., 2009, 48, 5392.

[15] S. Shi, J. Zhao, X. Geng, T. Yao, H. Huang, T. Liu, L. Zheng, Z. Li, D. Yang and L. Ji, "Molecular "Light switch'' for G-quadruplexes and i-moeity of human telomeric DNA" Dalton Trans., 2010, 39, 2490.

[16] K. E. Erkkila, D. T. Odom and J. K. Barton, Chem. "Recognition and reaction of metallointercalators with DNA" Rev., 1999, 99, 2777.

[17]. A. Hergueta-Bravo, M. E. Jime¢nez-Herna¢ndez, F. Montero, E. Oliveros and G. Rrellana, "Singlet oxygen-mediated DNA photocleavage with Ru(II) polypyridyl complexes"J. Phys. Chem. B, 2002, 106, 4010.

[18]. H. Y. Ding, X. S. Wang, L. Q. Song, J. R. Chen, J. H. Yu, L. Chao and B. W. Zhang, J. Photochem. Photobiol., A, 2006, 177, 286.

[19]. D. Lahiri, T. Bhowmick, B. Pathak, O. Shameema, A. K. Patra, R. S. amakumar and A. R. Chakravarty, "Anaerobic photocleavage of DNA in Red light by Cu(II) complexes.Inorg. Chem., 2009, 48, 339.

[20]. D. Lawrence, V.G. Vaidyanathan, B.U. Nair, J. "DNA binding studies of two mixed ligand complexes" Inorg. Biochem. 2006, 100, 1244 –1251.

[21]. F. Gao, H. Chao, F. Zhou, Y.-X. Yuan, B. Peng, L.-N. Ji, "DNA interaction of funtionalised Ru(II)complexes" J. Inorg. Biochem. 2006, 100, 1487 –1494.

[22] T. Hirohama, Y. Kuranuki, E. Ebina, T. Sugizaki, H. Arii, M. Chikira, P.T. Selvi, M. Palaniandavar, J. Inorg. Biochem. 2005, 99, 1205 –1219.

[23] X.-W. Liu, J. Li, H. Li, K.-C. Zeng, H. Chao, L.-N. Ji, J. Inorg. Biochem. 2005, 99, 2372 –2380.

[24] L.-F. Tan, H. Chao, Y.-J. Liu, H. Li, B. Sun, L.-N. Ji, Inorg. Chim. Acta 2005, 358, 2191 –2198.

[25] G.Siedle, B. Kersting, Z.Anorg. "DNA binding properties and nuclease activity of Cu(II) complexes" Allg. Chem 629 (2003) 2083

[26]. S.Anbu, M.Kandaswamy. P.Sathya Moorthy, M. Balasubramanian, MN. Ponnuswamy, "DNA binding studies of Cu(II) complexes using polyaza dinucleating ligands" Polyhedron. 28 (2009), 49-56.