Synthesis of New Cyclic Binuclear Copper (II) Complexes: DNA/BSA Binding and Cleavage Studies

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Abstract — **New series of macrocyclic copper(II) complexes have been synthesized by using 2,6-diformyphenol based mono nuclear copper(II) complexes with various diamines like 1,2-diamino ethane (L1), 1,3-diamino propane (L2), 1,2-diamino benzene (L3), 2-aminobenzylamine (L4), and 1,8-diamino naphthalene (L5). The complexes were characterized by elemental analysis and spectroscopic methods. Cyclic voltammogram of mononuclear copper(II) complex exhibit one quasi reversible reduction wave in the cathodic region and binuclear copper(II) complexes exhibits two quasi reversible waves. The DNA/BSA binding, DNA cleavage studies of these complexes were investigated. The binding propensities of the complexes toward calf thymus (CT DNA) have been investigated by spectroscopic methods (UV, Fluorescent and Viscosity measurements spectral studies). The binding constant (Kb) values in the range of from 0.19 X 105 M-1 to 0.25 X 105 M-1 and apparent binding constants (Kapp) are in range from 0.32 X 106 M-1 to 1.82 X 106 M-1 were measured in UV and Fluorescent methods. The cleavage activities are in the following order (6) > (1) > (2). The mechanistic investigation suggests that hydroxyl radical play a vital role in the cleavage process. All the copper(II) complexes (1-6) exhibit significant interaction with Bovine Serum Albumin (BSA), the results showed that the binding mechanism was static quenching process.**

Keywords- Macrocyclic copper(II) complexes, DNA/BSA binding studies, DNA cleavage studies.

I. INTRODUCTION

The field of bioinorganic chemistry is highly interdisciplinary research field, which deals with inorganic chemistry and biology, and put forth a new way for scientific research in coordination compounds.[1,2] The past few decades has demonstrated that the cancer can be effectively treated with surgery, chemotherapy and radiotherapy, which can significantly impact tumor growth and even produce cures when they are used either alone or in combination. Chemotherapy is a thriving area of research,[3,4]which was initially fueled by the discovery of the metallo pharmaceutical Cisplatin about 50 years ago, and is currently still one of the most widely, used anticancer drugs.[5] Due to their severe toxicity, their high activity is not always satisfactory and side effects are frequently encountered.[6,7] In this point of view, there is need for alternatives to Cisplatin, have consequently inspired further work towards the development of numerous non platinum metal based,[8] especially transition metal complexes, very quickly turned out to the interesting and attractive compounds in the development of anticancer drugs, and put forth a new way for scientific research in coordination compounds because of many different coordination geometries enable the synthesis of compounds with their stereochemistry and its chemical reactivity.[9,10] The geometry of metal ions complexes containing nitrogen or oxygen aromatic heterocyclic compounds as liands [11] and the oxidation state of the metal, those factors are control and regulate the biological activity of the metal-based drugs.[12] Copper based drugs have been now proposed as potential anticancer substances, demonstrating remarkable anticancer activity and showing general toxicity lower than platinum compounds. Many redox enzymes contain copper atoms bound to protein molecules like hemocyanins, superoxide dismutase, and blue copper proteins. In addition copper ions are found to present in the active sites of large number of metalloproteins, which involved in important biological electron transfer reactions as well as in the molecular oxygen redox reactions and also copper(II) complexes with amino acids are cited as having potent anti-inflammatory and anti-ulcer activity.[13, 14] The drug interactions at protein binding level significantly affect the apparent distribution volume and their elimination rate. Serum albumins are the most abundant protein in plasma. It plays a key role in storage and transport protein for numerous endogenous and exogenous compounds. In addition, binding of drugs with plasma proteins controls their free, active concentrations and provides a reservoir for a longer action, the binding of drugs is responsible for the protective role of albumin.

In this paper, we have described the synthesis and characterization of a series of cylic copper(II) complexes (Scheme 1). The reactivity's towards protein BSA of the acylic copper(II) complexes have been discussed. Nuclease activities of copper(II) complexes and mechanism of cleavage also studied.

II. EXPERIMENTAL

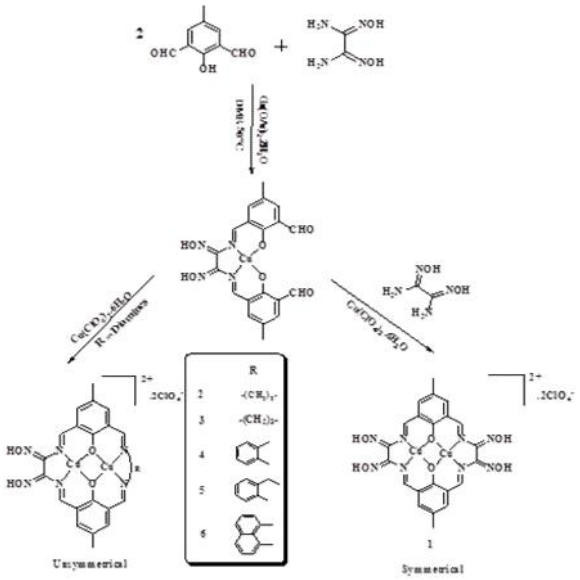
A. Materials and Methods

2,6-Difromylphenol, [15] 1,2-Bis(aminooxy)ethane[16] and precursor complexes [17,18] (PC) [Cu(sal-diamines)(py)](ClO4), (diamine- 1,2-diamino ethane(PC-I), 1,3-diaminopropane(PC-II), 1,2-diamino benzene(PC-III), 2-aminobenzylamine(PC-IV), 1,8-diamino naphthalene(PCV)) were prepared from the earlier reported methods. Tetra(n-butyl)ammoniumperchlorate (TBAP) was purchased from Fluka and recrystallized from hot methanol used as the supporting electrolyte in electrochemical measurement, (Caution! TBAP is potentially explosive and hence, care should be taken in handling the compound). All other chemicals and solvents were purified by reported procedures.[19]

Elemental analysis was carried out in Carlo Erba model 1106 elemental analyzer. FT-IR spectra were recorded in (4000 – 400 cm-1) 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 200–900 nm. Emission intensity measurements were carried out using Perkin Elmer LS-45 fluorescence spectrometer. Electrochemical measurements were performed using Electrochemical analyzer CHI 1008 using a three-electrode cell. Glassy carbon electrode is a working electrode with saturated Ag/AgCl electrode as the reference electrode and platinum wire acts auxiliary electrode. The concentration of all the complexes was make at 10-3 M. TBAP (10-1 M) was used as the supporting electrolyte to all electrochemical experiments. Electron spray ionization mass spectral (ESI-MS) measurements were performed by Thermo Finnegan LCQ-6000 Advantage Max-ESI mass spectrometer and acetonitrile as a solvent. Cyclic dichroism (CD) spectra were measured by JASCO J-715 spectropolarimeter.

B. Synthesis of copper(II) complexes

To a solution of 2, 6-diformyl-4-methyl phenol (3.0 g ; 18 mmol) in warm dimethyl formamide (30ml), 1, 2-Bis(aminooxy)ethane (0.84 g. 0.9 mmol) was added dropwise under constant stirring. Solid $Cu(OAc)_2.2H_2O$ (1.8 g, 0.9 mmol) was added and the solution was stirred at 60°C for 2 h. The resulting mononuclear complex [**CuL**] gets precipitated and the solid was separated out by filtration and washed with 2-propanol and diethyl ether.The binuclear Cu(II) complexes (1–6) were prepared from a general synthetic procedure. To vigorously stirred suspension of mononuclear complex CuL (0.565 g, 1.2 mmol) in methanol (25 ml), a methanolic solution containing Cu(ClO4)2.6H2O (0.44 g, 1.2 mmol) was added slowly and the mixture was stirred for 15 min to obtain a clear solution. Then the methanolic solution (5 ml) of corresponding diamine (0.14 g, 1, 2-diaminoglyoxime; 0.07 g ,1, 2-diamino ethane; 0.08 g, 1,3-diamino propane; 0.13 g, 1,4-diamino benzene; 0.15 g, 2-aminobenzylamine; 0.19 g, 1,8- diamino naphthalene; 1.2 mmol) was added drop wise to the above solution and refluxed for 3 h. The resulting solid was separated by evaporating the solution at room temperature and the resulting compound was washed with ether and dried under vacuum.



Scheme 1. Synthesis of new copper(II) complexes (1-6)

C. Protein binding studies

Binding nature of the copper(II) complexes (**1-6)** with BSA were recorded using fluorescence spectra from 300 nm to 500 nm at excitation wavelength 280 nm. BSA stock solution was prepared in 50 mM phosphate buffer (PBS) (pH = 7.2) and stored in 4 ºC. All the test samples were purging with nitrogen gas for 15 min to remove dissolved oxygen. Titrations were manually carried out using micropipette by the addition of complexes. The binding study of BSA with complexes are measured by UV spectrum which in the range of 200 nm to 500 nm. First record the spectrum of BSA (10 µM) and the peak was observed in the range of 280 nm and addition of the complex to equal molar to that of BSA solution.

III. RESULTS AND DISCUSSION

A. structural analysis of copper(II) complexes

The FT-IR spectra of the symmetrical binuclear copper(II) complexes (1-6) were carried out and sown in Fig. 91. The complexes showed the peak in the range 3440-3460 cm-1 indicating the presence of ν (O-H) of water molecules. The strong band observed for the complexes in the region around 1080-1110 cm-1 and sharp band in the region around 626 cm-1 could be due to the antisymmetric stretch and antisymmetric bending of perchlorate ions respectively. The entire spectrum shows asharp new peak in the range of 1620 to 1640 cm-1. The disappearance of the peak around 1680 cm-1 υ (C=O)this shows that the aldehyde group had been completely converted into imine groups in the schiff base condensation reaction. Further, the appearance of bands in the region of 1510-1540 cm-1 for binuclear complexes suggest that the presence of phenoxide bridging between the metal ions.

ESI-MS spectrum shows the molecular ion peak indicating the stability of the structure in a solvent medium. The Cu(II) complexes in acetonitrile shows the peak at (m/e) 617, 622 and 656 corresponds to molecular ions of (1), (5) and (6) respectively. The spectrum of the macrocylic copper(II) complex peak indicate that the complex (1) shows as the [M+1], the complex (2) shows as the [M+2] peak and the complex (6) shows as the [M+] peak. The ESI mass spectrum of the binuclear copper(II) complex indicating the structural orientation of the binuclear copper(II) complexes and ESI mass spectral data the symmetrical and unsymmetrical binuclear Cu (II) complexes confirm the proposed formula of the complexes.

The copper(II) complexes (1-6) show three peaks in electronic spectra. One is d-d transition in the range 560-620 nm, the peaks in the range of 320-410 nm is due to ligand-to-metal charge transfer transition (LMCT) and finallyan intense peak below 300 nm is assigned to the intra-ligand charge transfer [20, 21] transition (π–π*). An increase in λmax (red shift) value for d-d transition of copper(II) ion as increasing the chain length61was observed for copper(II) complexes, which indicates that the distortion of the coordination geometry around the metal ion increases.

Electrochemical characterization

The electrochemical behavior of macrocyclic binuclear copper(II) complexes (1-6)has been studied by cyclic voltammetry in dimethylformamide containing 10-1 M tetra(n-butyl)ammonium perchlorate and the data are summarized in Table 1. The cyclic voltammograms of the complexes were studies in the potential range of -0.4 to -1.4 V.

The reduction potential of the mononuclear copper(II) complex show from -0.94 V to -0.79 V. Macrocyclic binuclear copper(II) complexes show the voltammogram of Fig. 1 and Fig. 1b of two quasi-reversible reduction waves. The first reduction potential ranges from -0.79 V to -0.99 V and the second reduction potential ranges from -1.17 V to -1.32 V. Coulometric titration by potentiostatic exhaustive electrolysis performed 100 mV more negative to the first and second reduction waves consumed approximately one electron at each reduction ($n \approx 0.97$). so, the redox processes are assigned as follows,

The reduction waves of the binuclear copper(II) complexes are quasireversible in nature as evidence the complexes are satisfied the following conditions.

(i). The Epcand Epa values change with scan rate

(ii). ΔEp values increases with increasing the scan rate and that value more than 60 mV

(iii). Cathodic peak currents (Ia) are greater than the anodic peak currents (Ic).

Wherein all the binuclear copper(II) complexes (1-6) satisfied the above condition, so strongly suggest that the complexes are quasi reversible nature. All the binuclear copper(II) complexes show two-step one-electron, quasi-reversible reduction waves. Two different reduction waves of all the binuclear copper(II)

complexes occurred may be due to different compartment present in the binuclear complexes. The first reduction potential of Cu(II) ion present in the glyoxime moiety present in binuclear complexes, may be due to the high electronegative oxygen present in one compartment of binuclear copper(II) complexes [22] also supported the mononuclear copper(II) complex reduce the lower reduction potential range. So, strongly suggests that the lower reduction potential occurred only in the glyoxime moiety. The more negative potentials of second quasireversible peaks are assigned to reduction of copper(II) ion, present in the azomethine moiety. As the chain length of the alkylimine compartment increases, the entire macrocyclic becomes more flexible, which enables the easier reduction. As the size of macrocycle is increased, shifting of both first and second reduction potentials towards anodic is observed for the binuclear copper(II) complexes.

. Table 1 Electrochemical data of copper(II) complexes (1-6)

Complexes	E^1_{pc}/V	E^1_{pa}/V	$E^1_{1/2}$/V	ΔE^1/mV	E^2_{pc}/V	E^2_{pa}/V	$E^2_{1/2}$/V	ΔE^2/mV
Mononuclear	-0.94	-0.79	-0.87	150	-	-	-	-
1	-0.79	-0.65	-0.72	140	1.27	1.08	1.18	190
2	-0.9	-0.8	-0.85	100	-1.29	-1.1	-1.19	190
3	-0.83	-0.7	-0.76	130	-1.21	-1.06	-1.13	150
4	-0.99	-0.9	-0.94	90	-1.32	-1.24	-1.28	80
5	-0.89	-0.77	-0.83	120	-1.25	-1.07	-1.15	180
6	-0.82	-0.71	-0.76	110	-1.17	-1.04	-1.10	130

Measured by Clyclic voltammograms at 100 mV/s conditions: GCE working; Ag/AgCl reference electrodes and TBAP as a supporting electrolyte; [Complex] = 1 X 10^{-3} M; [TBAP] = 0.1 M.

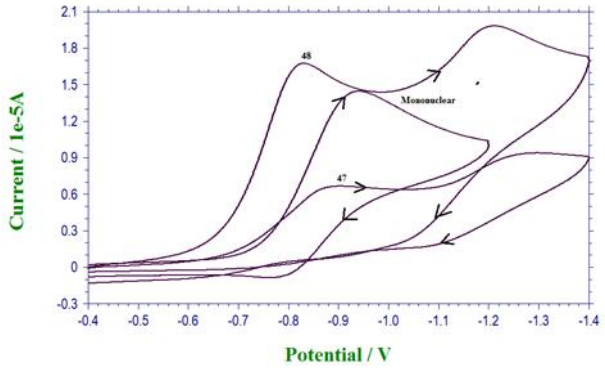


Fig. 1. Cyclicvoltammogram of binuclear copper(II) complexes (1-6)

B. Protein binding studies

(i) UV-vis absorption changes BSA-Cu(II) complexes

UV-vis absorption changes is a useful and very simple method do determine the structural changes and to know the complex formation. When addition of copper(II) complex to BSA to explore the structural changes in the conformation of the peptide backbone associated with the helix-coil transformation. Here, two types of quenching mechanism can occur, one is dynamic and another one is static quenching. For dynamic quenching mechanism, only the excited state fluorescence molecule undergoes change in the absorption spectra, while static quenching mechanism, complex formation between quencher and fluorophore molecule in ground state, therefore considerably change the absorption in UV-vis spectra. Fig 6. Shown change in absorption spectra for BSA and BSA-complex. The absorption spectra of BSA occurred at 278 nm, after addition complexes, the absorption has decreased with small red shift. The literature shows that [23]. the peak in the 278 nm region in the difference spectra of proteins is related to changes in the conformation due to exist as static interaction between BSA and copper(II) complexes due to the formation of new ground state complexes of the type BSA-complex[24]

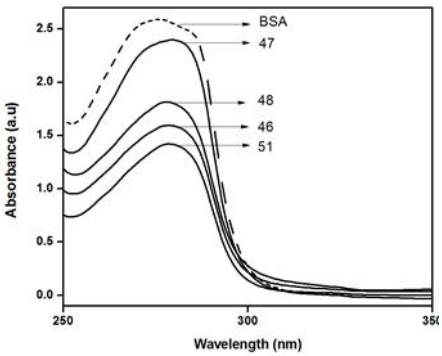


Fig. 6 UV-Vis absorption spectra of BSA in the absence and presence of complexes (1, 2, 3 and 6) Dashed line: the absorption spectrum of [BSA]. Solid line: the absorption spectrum of proteins in the presence of complexes at the same concentration, [BSA] = [Complex] = 10 µM.

(ii) Fluorescence quenching studies

Serum albumins are proteins involved in the transportation of essential trace metal ions and metal complexes with drugs in the circulatory systems. Fluorescence quenching studies has been widely used to study the interaction of complexes with BSA was studied from tryptophan emission-quenching experiments. When the excitation wavelength at 280 nm, the emission peaks for BSA located at 348 nm. Fluorescence quenching studies is decrease in fluorescence intensity and small red shift occurred regularly with increasing amounts of metal complexes are shown in Fig. 7(a, b, c) and that occurs by various types such as ground state complex formation, excited state reaction, energy transfer and collision quenching.
The fluorescence quenching can be described by Stern-Volmer relation:

$$I_0 / I = 1 + K_{SV}[Q]$$

WhereI_0 and I are the fluorescence intensities of the fluorophore in the absence and in the presence of quencher, Ksv is the Stern-Volmer quenching constant and [Q] is the quencher concentration. According to the equation; Ksv = Kq$\tau_{0,}$ where Kq is the quenching rate constant and τ_0 is the fluorescence life time of protein in the absence of quencher, which is about 10^{-8}s. All the values of Kq were much greater than the maximum scatter collision quenching constant of various quenchers (2 x 10^{10} M^{-1} s^{-1}). This value suggested that the quenching was a static process [22, 26]. For static quenching process, the relationship between the fluorescence intensity and the concentration of quencher can be usually described by the following Eq

$$\text{Log}\,[(F_0 - F)/F] = \text{Log}\,K_b + n\,\text{Log}\,[Q]$$

where in the present case, F_0 and F are the fluorescence intensities in the absence and the presence of quencher, [Q] is the concentration of quencher and K_b is the binding constant for the complex-protein interaction and n is the number of binding sites per albumin molecule, which can be determined by the slope and the intercept of the double logarithmic plot (Fig. 8) of log [(F_0-F)/F] vs log[Q] based on equation (1) The calculated value of quenching constant (Kq), binding constant (K_{bin}), and the number of binding sites (n) are listed in Table 2. The values of n are approximately equal to 1, indicates that there is single binding site in BSA for all the complexes. The order of magnitude of binding constants was around 10^5 indicating the existence of strong interaction between BSA and copper(II) complexes.

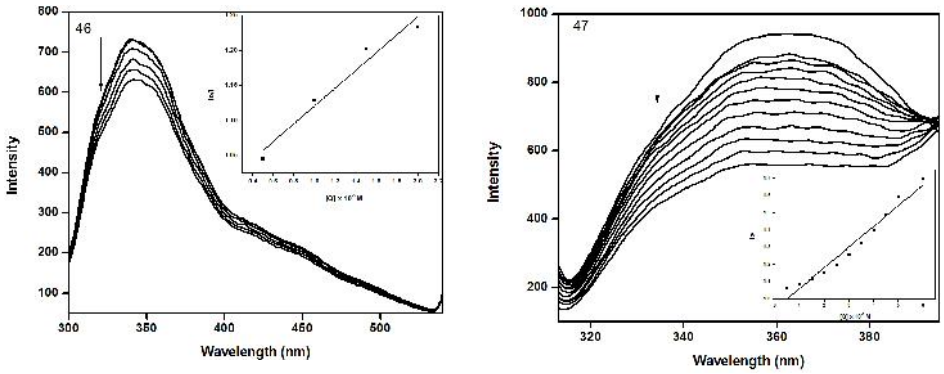


Fig.7a Changes in the fluorescence spectra of BSA upon increasing complexes(1) and (2) concentration at 300 K. the concentration of proteins is 1 µM and complex concentration was varied from 0.0 to 10 µM, pH = 7.2 and λex = 280 nm.

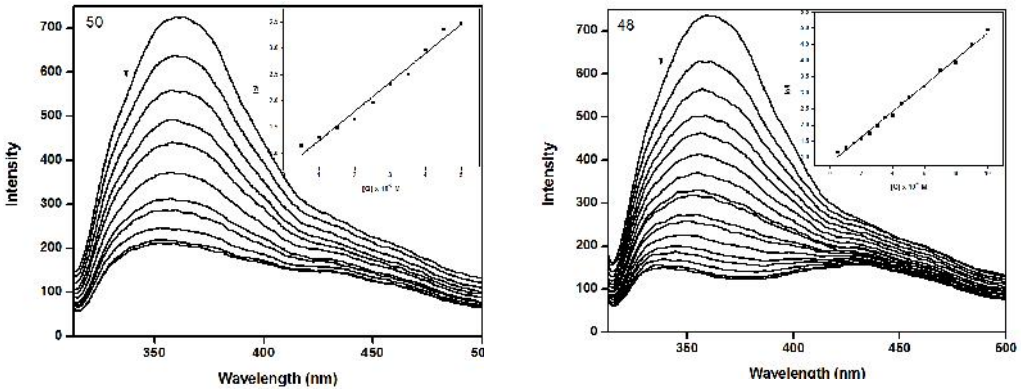


Fig.7b Changes in the fluorescence spectra of BSA upon increasing complexes(3) and (5) concentration at 300 K. the concentration of proteins is 1 µM and complex concentration was varied from 0.0 to 10 µM, pH = 7.2 and λex = 280 nm.

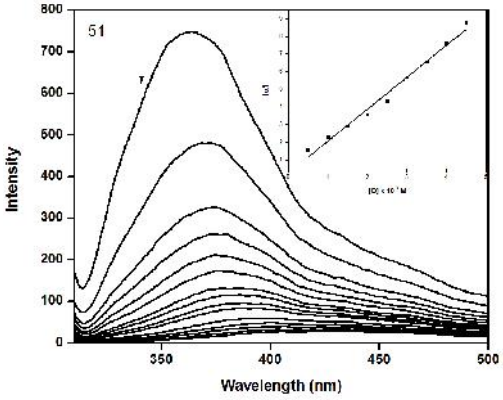


Fig.7c Changes in the fluorescence spectra of BSA upon increasing complex (6) concentration at 300 K. the concentration of proteins is 1 µM and complex concentration was varied from 0.0 to 10 µM, pH = 7.2 and λex = 280 nm.

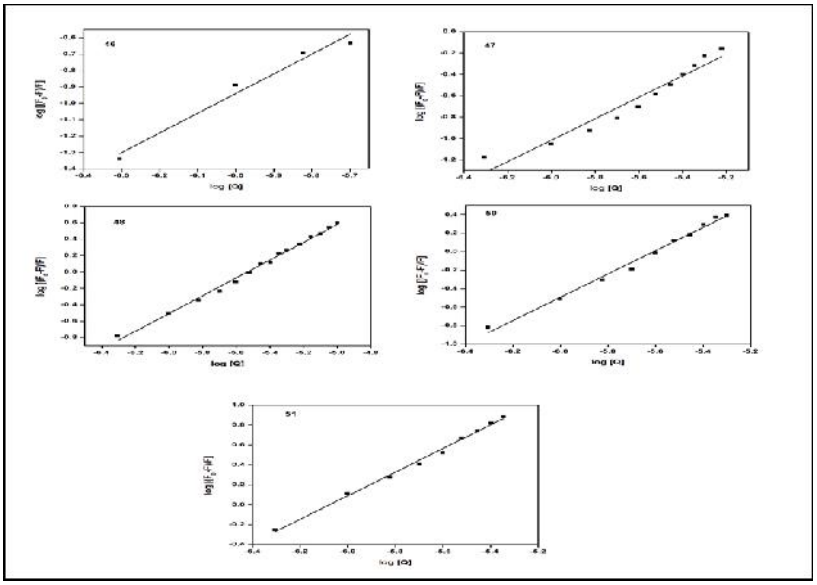


Fig.8 Double-logarithm plot determining binding constant and number of binding sites for Copper(II) complexes (1, 2, 3, 5 and 6) interaction with BSA (T= 298 K).

Table 2: The static binding constants (Kbin in M-1) and binding sites for the interaction of copper(II) complexes (1-6) with BSA at 298 K.

Complexes	K_{sv} (mol L^{-1})	Quenching rate constant Kq ($M^{-1}s^{-1}$)	K_{bin} (L mol^{-1})	The number of binding sites per BSA n
46	1.27×10^5	1.27×10^{13}	1.9×10^6	1.203
47	1.28×10^5	1.28×10^{13}	0.104×10^6	1.005
48	4.83×10^5	4.83×10^{13}	1.01×10^6	1.085
50	7.85×10^5	7.85×10^{13}	1.094×10^7	1.255
51	6.2×10^5	6.2×10^{13}	1.58×10^7	1.185

IV.CONCLUSION

A series of multidentate copper(II) complexes are synthesized and characterized and their BSA binding, The binding constant (Kb) values of the complexes (1) and (6) are 0.84 X 104(1), and 3.0X104 M-1(6) are calculated using absorption spectral method and apparent binding constant (Kapp) are calculated as 1.9 X 106 M-1(1) and 4.2 X 106 M-1 (6) by fluorescence spectral method. The excellent binding properties of complex (6) confirmed that the extending aromatic (naphthalene) moiety in the acyclic ring leads to maximum intercalative interaction compare to aliphatic (ethylene) moiety. Complex binds with BSA experimental (UV and fluorescence) results suggested that all the copper(II) complexes (1-6) could interact high affinity and quench the fluorescence of BSA through static quenching mechanism. According to the present work represent a good overall correlation between BSA binding activity for complex (6). Thus complex (6) better promising candidate to further design and develop new copper(II) based complexes, for a systematic assessment of their BSA binding activity, and their potential application as therapeutic agents

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