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A Study of Copper (II) Schiff Base Ligand Complexes

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Abstract - The palladium(II), copper(II), cobalt(III) and ruthenium (III) Schiff base complexes with the above-mentioned ligands (L1-L4), were summarised as dichloromethyl, ethanol/benzene medium, all of which are strong stable, long-term in air, characterised by different physicochemical technologies. The complexes are water insoluble but DMF and DMSO soluble. Yet ethanol and methanol are readily soluble in ruthenium and palladium complexes. The experimental evidence on the complex elemental analysis is well in line with that of the formulation being proposed. The observed molar conductance values are consistent with the non-electrolytic properties of the preparations except for the Co(III) complexes, which are electrolytes, for all the complexes of 10−3 M DMF solution. By analysing different physicochemical techniques, the structures of the prepared complexes are proposed.

Keywords - Copper (II), Schiff Base Ligand, Complexes, Experimental

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1. INTRODUCTION

Copper is an important micronutrient present in enzymes like Lysyl oxidase, dopamine β-monooxygenase, cytochrome c oxidase, superoxide dismutase and diaminase oxidase. The redox chemistry of copper made its applicability as catalytic cofactor to perform fundamental biological functions including oxygen transport and angiogenesis. Metal binding with biological macromolecules control the conformations and coordination environment. Transition metal complexes interaction with DNA provides a rational route towards the drug design and tools for molecular biology. Copper Schiff base complexes with tunable geometry could find its applications at cellular level acting as chemotherapeutic agents. Metal complexes bind to DNA non-covalently by various mechanisms; intercalation for a planar ligand, electrostatic interaction for cations, groove binding for sterically demanding ligand and partial intercalation for incompletely planar ligands. Biologically active molecules can significantly affect the cytotoxic activity of the metal complexes, in particular, nitrogen containing heterocyclic moieties. Benzimidazoles and their derivatives possess extensive biological activities and clinical applications and therefore contribute significantly in designing novel drug molecules.[1] Benzimidazoles are being structural analogs of naturally occurring nucleotides, allows them to interact easily with the biopolymers of the living systems, which makes them perfect candidates for the drug design and discovery. In recent years, copper complexes with mixed ligand like 1, 10-phenanthroline, show excellent anticancer property by inducing apoptosis due to their strong DNA binding/cleaving ability.

2. EXPERIMENTAL

1. Chemicals

All solvents and reagents were obtained from commercial sources and used without further purification unless otherwise stated. Copper chloride dihydrate and 1, 10-phenanthroline monohydrates were purchased from Sigma Aldrich Chemicals Pvt. Ltd. Bengaluru.[2]

2. Preparation of complexes (4a-4h)

All copper complexes (4a-4h) were synthesized from ligands L1-L4 in the ratio of 1:2 (M:L) and for the complexes in the ratio 1:1:1 (M:L:phen), with an auxillary ligand o-phenanthroline.

2.1. Preparation of complexes (4a-4d) in the ratio of 1:2

An ethanolic solution of $CuCl_2 2H_2O$ (1 mmol) was added slowly to a hot ethanolic solution of each of

the Schiff base ligand L1-L4 (2 mmol) separately. Thereaction mixture was refluxed for 4 h on a water bath. Then the solvent was evaporated to (1/3)rd of the volume. The solid complexes were obtained by adding n-hexane/ diethyl ether. Then solid was filtered off, washed with cold ethanol and dried over $CaCl_2$. The development of single crystal for each of these complexes was unsuccessful.

Complex 4a: yield: 75%. m.p>300 °C. IR v (cm-1): 1619 (HC=N), 3320 (NH, benzimidazole), ESI – MS m/z: 765.2130 [M]+, 767.7297 [M+2]+ .Anal. Calcdfor [C42H34CuN6O5] (%): C, 65.83; H, 4.47; N, 10.97; Found C, 64.41; H, 6.02; N, 9.91. Conductance (Λ, Ω-1 cm 2 mol-1): 3.4; μeff: 1.86 BM.

Complex 4b: yield: 80%. m.p>300 °C. IR v (cm-1): 1630 (HC=N), 3330 (NH, benzimidazole), ESI – MS m/z: 897.2181 [M]+ ,899.2181 [M+2]+ . Anal. Calcdfor [C44H40Cl2CuN6O7] (%): C, 58.77; H, 4.48; N, 9.35; Found C, 58.69; H, 4.42; N, 9.29. Conductance (Λ, Ω-1 cm 2 mol-1): 4.1; μeff: 1.85 BM

Complex 4c: yield: 73%. m.p>300 °C. IR v (cm-1): 1617 (HC=N), 3320 (NH, benzimidazole), ESI – MS m/z: 773.1026 [M]+ ,775.6078 [M+2]+ . Anal. Calcdfor [C40H28Cl2CuN6O3] (%): C, 61.98; H, 3.64; N, 10.84; Found C, 60.12; H, 4.54; N, 9.62. Conductance (Λ, Ω-1 cm 2 mol-1): 5.7; μeff: 1.78 BM.

Complex 4d: yield: 80%. m.p>300 °C. IR v (cm-1): 1625 (HC=N), 3325 (NH,benzimidazole), ESI – MS m/z: 921.0265 [M]+ , 923.2354 [M+2]+ . Anal. Calcd for [C42H32Br2CuN6O5] (%): C, 54.59; H, 3.49; N, 9.09;Found C, 53.80; H, 3.09; N,8.87. Conductance (Λ, Ω-1 cm 2 mol-1): 3.4; μeff: 1.98 BM.

2.2. Preparation of (4e-4h) in the ratio of 1:1:1

An ethanolic solution of $CuCl_2 2H_2O$ (1 mmol) was refluxed with a heterocyclic base 1, 10-phenanthroline monohydrate (phen) (1 mmol) in 10 mL of ethanol under stirring for 30 min. The resulting solution was then added with a hot ethanolic solution of each ligand L1-L4 (1 mmol) separately. The reaction mixture was refluxed for 4 h on a water bath and the solvent was reduced to (1/3)rd of the volume. The solid complexes were obtained by adding n-hexane. Then it was filtered, washed with cold ethanol and dried over $CaCl_2$.[3]

Complex 4e: yield: 73%. m.p>300 °C. IR v (cm-1): 1617 (HC=N), 3320 (NH,benzimidazole), ESI – MS m/z: 657.1214 [M]+ . 659.1587 [M+2]+ . Anal. Calcd for [C33H28ClCuN5O4] (%): C, 60.27; H, 4.29; N, 10.53; Found C, 59.39; H, 4.25; N,9.00. Conductance (Λ, Ω-1 cm 2 mol-1): 9.12; μeff: 1.79 BM.

Complex 4f: yield: 83%. m.p>300 °C. IR v (cm-1): 1625 (HC=N), 3325 (NH, benzimidazole), ESI – MS m/z: 705.1523 [M]+ .707.1567 [M+2]+ . Anal. Calcdfor [C34H29Cl2CuN5O4] (%): C, 57.84; H, 4.14; N, 9.92; Found C, 57.80; H, 4.09; N, 9.87. Conductance (Λ, Ω-1 cm2 mol-1): 8.4; μeff: 1.80 BM.

Complex 4g: yield: 80%. m.p>300 °C. IR v (cm-1): 1630 (HC=N), 3330 (NH,benzimidazole), ESI – MS m/z: 661.0754 [M]+ . 663.0657 [M+2]+ . Anal. Calcdfor [C32H25Cl2CuN5O3] (%): C, 58.06; H, 3.81; N, 10.58; Found C, 57.69; H, 3.72;N, 9.59. Conductance (Λ, Ω-1 cm 2 mol-1): 8.1; μeff: 1.85 BM.

Complex 4h: yield: 73%. m.p>300 °C. IR v (cm-1): 1617 (HC=N), 3320 (NH,benzimidazole), ESI – MS m/z: 735.5732 [M]+ . 737.5626 [M+2]+ . Anal. Calcdfor [C33H27BrClCuN5O4] (%): C, 53.82; H, 3.70; N, 9.51; Found C, 52.99; H, 3.25;N, 8.71. Conductance (Λ, Ω-1 cm 2 mol-1): 7.7; μeff: 1.83 BM.

3. PHYSICAL MEASUREMENT

The synthesized copper complexes were characterized by analytical, physical and spectral techniques.

3.1. Magnetic susceptibility measurements

The magnetic susceptibility measurements of the complexes were carried out in order to find out the effective magnetic moment per each metal in the complexes. The number of unpaired electrons possessed by the metal ion can be determined from the effective magnetic moment of the metal ion. On the basis of number of unpaired electrons, it is possible to infer the valence state of the metal ion in the complex and further if there are more than three d-electrons and the complex is of octahedral stereochemistry whether the bonding is of spin-free or spin-paired type.[4]

$$\mu_{eff} = [n(n+1)]^{1/2} \text{ B.M}$$

The magnetic susceptibility of the solid complexes was determined by the Gouy method at room temperature (26 ± 2 °C) using $Co(SCN)_4Hg$ as the calibrant. The effective magnetic moment per metal atom was calculated from the expression

$$\mu_{eff} = 2.84 [\chi_m T]^{1/2} \text{ B.M}$$

where χm is the molar susceptibility of the complex obtained after applying the diamagnetic corrections by the use of Pascals constants for other atoms and groups in the complex.

3.2. EPR spectra

EPR spectral technique is one of the reliable methods for the determination of geometry and electronic structure of the metal complexes. The EPR spectrum of each complex was scanned on a JEOL X-Band at 77 K under nitrogen, using Tetracyanoethylene (TCNE) as the g- marker and the sample concentration ca. 1 × 10–3 moldm–3 was maintained. The EPR spectrum of complexes contains information about the electronic environment around the metal center. Ligand field

theory was used to interpret the EPR parameters of the metal complexes. The presence of unpaired electrons and the degeneracy of the d-orbitals give rise to the anisotropic g‖ and g⊥ values.[5]

4. RESULTS AND DISCUSSION

All the prepared compounds are stable at room temperature, insoluble in water but slightly soluble in methanol and ethanol and completely soluble in DMF and DMSO. Elemental analysis of the complexes indicates the stoichiometry to be 1:2 (metal: ligand) for 4a – 4d, whereas 1:1:1 (metal: ligand: phen) for 4e – 4h.[6]

4.1 Magnetic susceptibility measurements

The magnetic susceptibility studies from the literature show that Cu(II) complexes possess paramagnetic in nature. A mononuclear distorted octahedral structure for copper(II) is assigned for 4a-4h as predicted by the μeff value for these complexes are found to be in the range of 1.78 - 1.98 B.M. which is higher than the spin-only value of 1.73 BM. This shows that the complexes have distorted octahedral geometry around the central metal ion with d 9 electronic configuration.

4.2 EPR spectra

EPR spectra of polycrystalline compounds 4a-4h were recorded on X-band at a low temperature of 77 K in DMSO. The representative EPR spectra of copper complexes 4b, 4c, 4f and 4g are as, copper(II) has one unpaired electron in its 3d orbital, with effective spin S= ½, which is equal to the actual spin of the free electron and I=3/2 for Cu2+ . The spectra show four well-resolved peaks inlow field region. The copper complexes exhibit the g|| and g⊥ value in the range 2.153-2.223 and 2.046-2.074, respectively, and g‖ > g⊥ > 2.0023. These values are indicate that the ground state of Cu(II) is predominantly dx 2 –y 2 with d 9 configuration. The values of the exchange coupling parameters obtained by the expression G = g‖ - 2.0023/g⊥ – 2.0023 lies at 2.71-3.84, estimated from low-temperature EPR spectra of the complexes, were ~4.0, suggesting the presence of exchange interactions in these complexes in solution at LNT (liquid nitrogen temperature). These EPR parameters are characteristic of isolated mononuclear Cu(II) ions located in octahedral site.[7]

Table 1: EPR parameters of copper complexes

Sl No	Complex	g‖	g⊥	G	g_{avg}	A‖	α^2
1	4a	2.184	2.069	2.71	2.107	170.12	0.78
2	4b	2.128	2.050	3.62	2.102	152.5	0.75
3	4c	2.167	2.052	3.30	2.090	181.42	0.729
4	4d	2.153	2.055	2.84	2.087	196.54	0.758
5	4e	2.221	2.046	3.05	2.122	176.25	0.782
6	4f	2.210	2.066	3.84	2.162	169.3	0.69
7	4g	2.214	2.059	3.66	2.110	184.20	0.788
8	4h	2.223	2.074	3.06	2.123	176.45	0.781

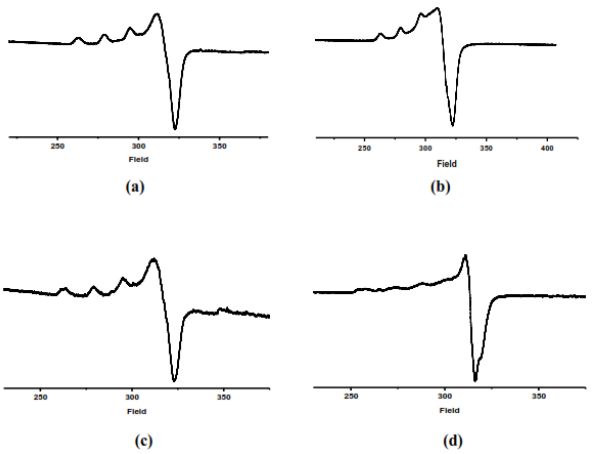

Figure 1: EPR spectra of (a) 4b, (b) 4c, (c) 4f, (d) 4g

5. BIOLOGICAL ACTIVITY

5.1. Antimicrobial activity

In vitro antimicrobial activity of the synthesized copper Schiff base complexes were performed by the well diffusion method. In this study, various concentrations of metal complexes were treated with four different types of bacteria and two fungi using ampicillin and fluconazole as the reference antibiotic and antifungal drug, respectively as a positive control and DMSO was used as a negative control. After inoculation, the plates were incubated and the agar plates were assessed for the zone of inhibition. A significant range of zone of inhibition was observed around individual wells and the results are tabulated in Table.[8]

Table 2: Results of Antimicrobial activity of copper(II) complexes

Compound	Zone of inhibition (mm)[a]					
	Bacteria				Fungi	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeroginosa*	*A.niger*	*A.flavus*
4a	12	13	17	15	19	17
4b	15	14	13	12	20	19
4c	16	15	18	16	22	20
4d	18	19	20	18	25	23
4e	14	16	17	21	23	21
4f	16	15	18	15	20	19
4g	18	16	19	21	25	22
4h	**20**	**22**	**24**	**27**	**28**	**25**
Ampicillin	24	25	27	29	--	--
Fluconazole	--	--	--	--	37	34

5.2. Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) value of the synthesized compounds 4a-4h was determined to know its susceptibilities for all strains of bacteria and fungi under study. The concentration of Cu(II) complexes at which there is no further growth of bacteria was observed and it was accounted as the

MIC values. Of all the complexes under study, 4h was shown to have lowest MIC value. A comparison of MIC values of the copper Schiff base complexes with standard drugs against microbial strains are presented in Table[9]

Table 3: Minimum inhibitory concentration results of 4a-4h (µg/mL)

Compound	Range of concentration (µg/mL)[a]					
	Bacteria				Fungai	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeroginosa*	*A.niger*	*A.flavus*
4a	90	85	90	90	95	90
4b	80	75	80	70	90	95
4c	60	65	70	65	85	85
4d	50	65	60	60	80	75
4e	85	80	85	90	90	85
4f	75	70	80	75	80	85
4g	55	75	60	55	75	70
4h	**45**	**50**	**55**	**50**	**65**	**60**
Ampicillin	15	15	15	15	--	--
Fluconazole	--	--	--	--	15	15

5.3. DNA interaction Studies

5.3.1. DNA Cleavage

DNA is the primary target for monitoring the antitumor efficacy, in the present work has examined the extent of DNA cleavage by the synthesized compounds using pUC 18 DNA. It is well known that the chemical nuclease activity is measured by relaxation of super coiled pUC 18 DNA to nicked and /or linear form. It should be informative that the excess stoichiometry of supercoiled DNA to metal complex causes more damage to DNA, probably due to increase in the amount of DNA bound complex. However, the substantial amount of unbound copper complex in the medium cannot cleave DNA; it is because the DNA cleavage is controlled by the formation of hydroxyl radical furnished due to the interaction of metal complex with an oxidant.[10]

5.4. DNA binding study

5.4.1. Electronic absorption titrations

The interaction of DNA with biologically active small molecule has been an interest of current research. The different binding mode to DNA gives an insight in understanding their biochemical mode of action of metal complexes. To account for the binding ability and nature of binding to CT-DNA, UV-visible and ethidium bromide displacement assay were employed. The UV-visible absorption spectra of all the synthesized compounds, in the absence and presence of DNA at increasing concentration.

In the UV region, the compounds represent two bands at 220 and 270 nm, which can be attributed to the π→π* transition of the benzimidazole Schiff base and 1,10-phenanthroline moiety. The absorption intensity showed hyperchromism on increasing the concentration of DNA from 0 to 25 µM, similar hyperchromism wasreported by some of the relevant literature bearing NH and OH groups. The binding constants (Kb) of compounds 4a-4h are found to be 3.04 × 104 , 2.20 × 104 , 4.70 × 104 , 6.02 × 104 , 6.42 × 104 , 5.79 × 104 , 8.42 × 104 and 9.82 × 104 M -1 , respectively. The obtained Kb values are lower than those of classical intercalator, ethidium bromide (EB) which was found to be the order of 106 -107 M -1 . Hence, by comparing the Kb values, it can be concluded that compounds 4a, 4b, 4c and 4d are bind to DNA through groove binding showing hyperchromicity with no sharp shift in the λmax. However, in the case of compounds 4e, 4f, 4g and 4h the absorption bands shows hyperchromicity with a slight red shift in λmax. Although, a slight red shift is expected for binding between aromatic chromophore with DNA base pairs, which is an indicative of the classical intercalating mode of binding. But, the absence of hypochromism in the absorption spectra ruled out the presence of complete intercalation. This indicates that the complexes 4e, 4f, 4g and 4h are bind through partial intercalation.

5.4.2. EB–DNA quenching assay

All the synthesized complexes are non-emissive in nature. Hence, ethidium bromide displacement experiments were conducted. The EB fluorophore, on the addition of nucleic acid, the fluorescence intensity was enhanced due to interaction with DNA. After the addition of the second molecule, that binds to DNA decreases the binding sites available for EB leading to the quenching of fluorescence intensity. In a competitive binding experiment, DNA was pretreated with EB was excited at λex 490 nm and λem 622 nm. The emission spectra of EB bound DNA was recorded both in the absence and presence of increasing amounts of prepared complexes. There is a concur reduction in the fluorescence emission intensity was observed on the addition of complex to EB bound DNA. Fluorescence quenching can be explained by displacement of ethidium bromide or by accepting the excited state electron from EB.

The quenching ability for complex was evaluated by Stern-Volmer constant Ksq, which can be determined by using the following equation:

$$I_O / I = 1 + K_{sq}[Q]$$

where IO and I correspond to the fluorescence intensities in the absence and presence of complexes, respectively, and r represents the ratio of total concentration of complexes to the CT-DNA, Ksq is a linear Stern-Volmer constant can be calculated from the slope of IO / I v/s [Q] inset plot.

The quenching plots have illustrated that the quenching of EB bound to DNA by synthesized compounds is in good agreement with the linear Stern-Volmer equation. The Stern-Volmer constants obtained from the slope of the graphs are tabulated. Higher quenching efficiency of 4e, 4f, 4g and 4h may be due to their interaction with DNA through additional planar aromatic base phen moiety, so releasing some free EB from the EB–DNA complex. From the UV-absorption titration and fluorescence quenching emission of complexes against CT-DNA, are concluded that 4g and 4h are found to be good DNA binding agents.

Table 3: Intrinsic binding constant (K_b) and Stern-Volmer constant (Ksv) of synthesized compounds

Sl. No	Compound	$K_b(M^{-1})$	$K_{sv}(M^{-1})$
1	4a	3.03×10^4	2.49×10^4
2	4b	2.20×10^4	1.46×10^4
3	4c	4.70×10^4	2.51×10^4
4	4d	6.02×10^4	2.88×10^4
5	4e	6.42×10^4	3.64×10^4
6	4f	5.79×10^4	2.88×10^4
7	4g	8.42×10^4	4.21×10^4
8	4h	9.82×10^4	4.89×10^4

5.4.3. Viscosity measurements

To further verify the nature of binding, the viscosity measurements were done. The relative viscosity was calculated for all the complexes 4a-4h, at a [complex]/[DNA] (r) ratio of 0.00 to 0.40 with an interval of 0.1. Representative plots of η/ηo vs [complex]/DNA. For 4e and 4f as can be seen, there is an initial decrease in viscosity with an increasing value of r = 0.0 to 0.20 indicate the non-intercalative interaction of the complex with DNA results in kink or bend in the DNA strand and hence, leads to diminishing in its effective length. Later increase of viscosity with increasing value of r = 0.2 to 0.4 which indicates a partial intercalative binding mode of the complex and supports an electrostatic binding mode together with some partial intercalative interactions for 4e and 4f. This is probably related to the molecular structures of the complexes, due to the existence of planar 1, 10-phenanthroline and groove binder benzimidazole. While for the 4c and 4d, they exert essentially no effect on DNA viscosity at low binding ratios from 0.0 to 0.2, but upon further increasing value of r = 0.2 to 0.4, the DNA viscosity decreases showing only an electrostatic interaction of complex with DNA. The experimental results are suggested that 4c and 4d could bind DNA in more of a groove than the intercalation and are consistent with the results obtained from the above experimental studies.

6. CONCLUSION

In the present investigation, synthesis, characterization, antibacterial and DNA binding and cleavage of Cu(II) complexes 4a-4h were carried out. The structure and bonding in complexes were understood by electronic absorption spectral data, IR techniques and various physicochemical methods. Electronic spectra and magnetic measurements are indicate that the distorted octahedral geometry for Cu(II) complexes. In vitro CT-DNA binding studies reveal that the complexes bind to the DNA helix via groove binding. The nuclease-like catalytic activity of a representative of the synthesized copper(II) complexes was investigated under aerobic conditions at room temperature. The obtained experimental observations are demonstrated that the complexes 4g and 4h have a promising ability toward the cleavage of the plasmid DNA oxidatively.

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