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Research Article

EVALUATION OF THE PHARMACOLOGICAL PROPERTIES OF SCHIFF BASE MIXED LIGAND Cu(II), Co(II) AND Ni(II) COMPLEXES DERIVED FROM (E)-3-((2-(1H-PYRROL-1-YL)PHENYL)IMINO)INDOLIN-2-ONE

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ABSTRACT

The coordination chemistry of (E)-3-((2-(1H-pyrrol-1-yl)phenyl)imino)indolin-2-one(L¹) and (E)-3-((2-(1H-pyrrol-1-yl)phenyl)imino)indolin-2-one(L¹) benzo[d]imidazol-2-yl)phenyl)imino)indolin-2-one(L²) has been investigated with the Cu(II), Co(II)and Ni(II)ions. The donor atoms and the plausible geometry of the complexes were examined by means of elemental analyses, molar conductance, magnetic moment, UV- Vis, IR, Far-IR and ESR spectra. The IR spectra results display the binding mode of the ligand involving azomethine nitrogen and carbonyl oxygen atoms. The electronic spectral and magnetic moment results indicate that Co(II) and Ni(II) complexes have octahedral geometry, while Cu(II) complex is distorted octahedral. The in-vitro biological screening effects of the investigated compounds were tested against bacterial species (Bacillus subtilis, Salmonella typhi) and fungal strains (Aspergillus niger, Candida albicans) by disc diffusion method. A comparative study of inhibition values result show that the metal complexes were found to be more active than the ligand. In addition, DNA cleavage studies for the newly prepared compounds were also studied. These studies indicate that the DNA cleavage efficacy increased in the complexes relative to the parental ligand.

Keywords: Schiff base, Isatin, 1-(2-aminophenyl)pyrrole, 2-(2-aminophenyl)benzimidazole, Antimicrobial and DNA cleavage

1. INTRODUCTION

Schiff bases provide possible sites for bio-chemically active compounds and have been studied widely over the past decade. The field of mixed ligand complexes was fast emerging account of the wide variety of potential structures for the ligands reliant upon the aldehydes and amines [1]. The Schiff base mixed-ligand complexes having N and O donor atoms are important due to their extensive antifungal, antibacterial, and anticancer activity [2]. Furthermore, mixed-ligand complexes are mostly found to be more biologically active than the ligand itself and its binary complexes [3, 4].

Isatin possess an indole ring structure, it is a flexible lead molecule common to many pharmaceuticals and heterocyclic natural products of biological interest [5]. The chemical versatility of Isatin motivated the prevalent use of this compound in biological synthesis [6]. Isatin Schiff bases were reported to exhibit a wide range of biological and pharmacological activities such as antibacterial, antifungal, antiviral, anti-HIV, anticancer, anti-tubercular, anticonvulsant, and antihelminthic

activities [7-9]. The interactions between Isatin Schiff base metal complexes and DNA have gained much attention due to their possible applications in cancer therapy [10-12]. From the literature survey, we find that a vast number of metal complexes because of their redox properties have been used as cleavage agents for DNA.

In view of the above facts, we report the evaluation of pharmacological properties of novel Schiff base ligand derived from the condensation of Isatin, 1-(2aminophenyl) pyrrole, 2-(2-aminophenyl)benzimidazole and its Co(II), Ni(II) and Cu(II) complexes. The main target of the present article is to examine their antimicrobial activity and DNA cleavage of Schiff base mixed ligand complexes.

2. EXPERIMENTAL

All the reagents were Aldrich products and were used as supplied. IR spectra were recorded on Thermo Nicolet (Avatar 370 model) instrument in the range of 4000-200 cm⁻¹ using KBr discs. Electronic spectra were obtained with a Perkin-Elmer Lambda 40(UV-Vis) spectrophoto-meter using DMF in the range of 200-800 nm. The molar conductance measurements were carried out at room temperature using Elico model conductivity meter. The ESR spectra of powder samples were recorded by means of E-112 ESR Spectrometer, X-band spectrometer (9.1 GHz) with 100 KHZ modulation frequency. ¹H NMR signals were obtained from Bruker Avance III, 400MHz model spectrometer. The Redox nature of the complex in DMF was measured using CHI-760 Electrochemical Analyzer.

2.1.Synthesis of (E)-3-((2-(1H-pyrrol-1-yl) phenyl) imino)indolin-2-one (L¹)

The solution of Isatin (1 mol in 20 mL methanol) was mixed in 1-(2-aminophenyl)pyrrole (1 mol in 20 mL methanol). Then this mixture was refluxed at 70°C for about 3hrs. A yellow precipitate solution was obtained which was allowed to cool at room temperature. The volume of the solvent was reduced by slow evaporation which led to the formation of a yellow solid product and product was washed with ethanol then dried in air. Schematic route for synthesis of (L¹) is given in scheme 1.

2.2.Synthesis of (E)-3-((2-(1H-benzo[d]imidazol-2-yl)phenyl)imino)indolin-2-one(L²)

To a solution of 1H-indole-2,3-dione (1mmol) in methanol, 2-(2-aminophenyl)benzimidazole (1 mmol) in methanol, was added dropwise. The above mixture was refluxed for about 3hrs. Then the reaction mixture solvent was kept for slow evaporation and cooled at room temperature. The yellow precipitate was filtered and then washed with ethanol. Schematic route for synthesis of (L^2) is given in scheme 2.

2.3.Synthesis of Schiff base mixed ligand Metal (II) Complexes

1 mmole of metal salt in methanol (20 ml) were mixed and stirred with 1 mmole Schiff base ligand (L^1) in warm methanolic solution (20 ml) in round bottom flask followed by the addition of ligand (L^2). Resulting reaction mixture was refluxed for 3 hours on water bath. Dark coloured complex was obtained and allowed for slow evaporation. Then washed with sufficient quantity of ethanol to dryness and dried in vacuum desiccators. Schematic route for synthesis of mixed ligand complexes are shown in scheme 3.



(E)-3-((2-(1H-benzo[d]imidazol-2-yl)phenyl)imino)indolin-2-one

Scheme 2: Structure of Schiff base Ligand (L²)



ML¹L²

Scheme 3: Synthesis of Schiff base mixed ligand complexes [ML¹L²]

2.4. Anti-microbial Activity

The ligands namely L^1 , L^2 and their complexes were screened for their antibacterial activity against *Bacillus Subtilis, Salmonella typhi* and antifungal activity against *Aspergillus niger, Candida albicans.* The standard drugs Chloramphenicol and Fluconazole were also tested for their antimicrobial activities at the same concentration (1 mg/ml) and compared with tested compounds.

2.5. DNA Cleavage Studies

The DNA cleavage ability of Co(II), Ni(II) and Cu(II)complexes against pUC18DNA was monitored with the aid of agarose gel electrophoresis method. Mixed ligand complexes are able to convert super coiled DNA into open circular DNA and cleavage activities of complexes are obviously depends on concentrations. The cleavage efficacy of the metal complexes compared to that of the control experiments is due to their capable DNA-binding ability.

3. RESULTS AND DISCUSSION

The Schiff base ligands (L^1, L^2) were yellow in colour, but the prepared metal complexes of these ligands vary in colour depending on the metal ions. The elemental analysis of the Schiff base mixed ligand and its complexes is listed in Table 1. The data obtained was in a good agreement with the theoretical values and show the formation of 1:1:1 [M: L^1 : L^2] ratio where M = Co (II), Ni (II) andCu (II) The metal complexes were found to be stable in air and insoluble in water, but soluble in DMF, DMSO and Chloroform. The observed values of molar conductance for the mixed ligand complexes (measured in 10^{-3} M DMF) are lower and thus non-electrolytic nature of the mixed ligand complexes was confirmed [13].

3.1.IR Spectra

In coordination chemistry, IR spectra provide a lot of valuable information about the nature of functional group attached to the metal atom [14]. The structure verification of metal complexes can be easily attained by comparing the IR spectra of the free ligands with their metal complexes. The IR spectrum of the ligands (L^1) and (L^2) are shown in Fig.1 and 2. Such comparison exposed that the characteristic absorption band appearing in free ligands spectra at 1616-1631 cm⁻¹ due to the azomethine group (C=N) is shifted to lower or higher wave numbers by 2-15 cm⁻¹ in the complexes spectra,

indicating coordination through the azomethine nitrogen [15]. The Schiff bases and complexes also display bands at 1725-1733 cm⁻¹ due to the Carbonyl (C=O) group of isatin moiety, indicating the participation of the oxygen atom of carbonyl group in coordination to the metal ion. Moreover, free ligands and metal complexes show a broadband around 3125-3350 cm⁻¹ which can be

attributed to N-H stretching vibration of isatin and benzimidazole moiety [16]. The IR spectra of the metal complexes also show some new bands in the 518-532, 435-480 and 330-361 cm⁻¹ region, which may probably due to the formation of (M–O) [17], (M–N) [18] and (M-Cl) [19] bands respectively.

Table 1: Analytic	cal data of the Schiff	base mixed ligand	and its metal complexes

Compounds	Molecular Formula	M.Wt	Yield -	Calculated/found%					
Compounds				С	Η	Ν	0	Cl	Μ
L ₁	$C_{18}H_{13}N_{3}O$	287.32	85	(75.25)	(4.56)	(14.63)	(5.57)	-	-
				75.26	4.52	14.63	5.57		
L ₂	$C_{21}H_{14}N_4O$	338.36	80	(74.54)	(4.17)	(16.56)	(4.73)	-	-
				74.55	4.14	16.56	4.73		
CuL ₁ L ₂	$C_{39}H_{27}CuN_7O_2cl_2$	758.09	82	(61.62)	(3.58)	(12.90)	(4.21)	(9.33)	(8.36)
				61.73	3.56	12.92	4.22	9.24	8.38
CoL_1L_2	$C_{39}H_{27}CoN_7O_2cl_2$	754.09	75	(62.00)	(3.60)	(12.98)	(4.24)	(9.39)	(7.80)
				62.06	3.58	12.99	4.24	9.29	7.81
NiL ₁ L ₂	C ₃₉ H ₂₇ NiN ₇ O ₂ cl ₂	753.10	70	(62.02)	(3.60)	(12.98)	(4.24)	(9.39)	(7.77)
				62.14	3.58	13.01	4.24	9.30	7.79



Fig. 1: IR Spectra for of Schiff base L¹



Fig. 2: IR Spectra for of Schiff base L²

3.2. Electronic spectra and magnetic studies

The UV spectra of the Schiff base ligands and their complexes were recorded in freshly prepared DMF as a solvent (Fig.3 & 4). In electronic spectrum, the absorption band observed around270-290 nm which is assigned to benzene π - π * transitions and 335-361nm which is attributed to $n-\pi^*$ transitions of C=N chromophore within the Schiff base molecule $(L^1 \& L^2)$. On complexation this band was shifted to lower or higher wavelength region showing the coordination of azomethine nitrogen to the central metal ion. The electronic spectrum of the [CuL¹L²]Cl₂ complex shows a broad band at 609 nm which can be assigned to attributed to the ${}^{2}Eg \rightarrow {}^{2}T_{2}g$ transition, which is compatible with these complexes having an octahedral structure. Similar structural features were assigned for other mixed ligand Co(II) and Ni(II) complexes.







Fig 4: UV Spectra of Schiff base L²

Magnetic susceptibility study of the present $Cu[C_{39}H_{27}N_7O_2]cl_2$ gives a magnetic moment value of 1.75 BM at room temperature, which are consistent with

an octahedral structure. The $Co[C_{39}H_{27}N_7O_2]cl_2$ complex has a magnetic moment value of 4.45 BM, which is in agreement with the reported value for octahedral. The Ni[C_{39}H_{27}N_7O_2]cl_2 complex reported herein has a room temperature magnetic moment value of 3.21 BM which is within the normal range observed for octahedral Ni(II) complex [20-22].

3.3. ¹H NMR Spectra

The ¹H NMR spectra of ligands $(L^1 \& L^2)$ were recorded in DMSO-d6. The proton NMR spectrum of both ligands is shown in Fig.5 & 6. The signals due to N-H proton appeared as singlet in the range 10.2-11.05 ppm for Schiff base $(L^1 \& L^2)$ corresponding to isatin and benzimidazole ring. The spectrum of free ligands, exhibited a signals in 6.2-7.8 ppm region which may be assigned to aromatic protons [15, 23]. The inferences drawn from these studies provide further support to the mode of bonding discussed in their IR spectra.



Fig. 5: ¹H NMR Spectra of Schiff base L¹



Fig. 6: ¹H NMR Spectra of Schiff base L²

3.4. Cyclic Voltammetry

The electrochemical study is the most effective and versatile methods accessible for study of redox systems.

The redox behaviour of Cu(II), Co(II) and Ni(II) complexes has been studied by cyclic voltammogram in DMF using 0.1 M Tetrabutyl ammonium perchlorate (TBAP) as supporting electrolyte. The voltammograms of the mixed ligand Schiff base complexes contains two peaks due to oxidative nature of organic molecule and reductive nature of azomethine group. The Cu(II) complex shows an cathodic peak at 0.54 V which is attributed to one electron reduction of Cu(II)/Cu(I) couple and anodic peak of 0.71 V indicates the oxidation process of $Cu(I) \rightarrow Cu(II)$. The Co(II) complexes exhibit well defined redox process corresponding to quasi reversible Co(II)/Co(I) couple with a peaks at Epa=0.96 V, Epc=0.64 V. The anodic peak at Epa= 0.75 V versus Ag/AgCl and the associated cathodic peak at Epc= 0.840V correspond to the Ni(II)/Ni(I) couple which provides evidence for quasi reversible in nature. It has been shown that the formal redox potential dependent on factors such as coordination number, hard/soft nature of the ligands and bulkiness of the ligands [24].

3.5. ESR studies

Based on hyperfine structures, the ESR spectrum for metal complexes provides information about the geometry, nature of coordinating sites of the Schiff base and the metal. The Schiff base mixed ligand Cu(II) complex display a small broad signal with g^{\parallel} at 2.08 and g^{\perp} at 2.03.Hence the unpaired electron lies in the dx²-y² orbital with $g^{\parallel} > g^{\perp} >$ ge (2.0027 free spin value) [25]. This confirms Cu(II) complex possess a distorted octahedral geometry.

3.6. Antimicrobial activity

In-vitro antifungal and antibacterial screening results are shown in Fig.7 and 8. Due to chelation, the metal complexes show prominent activity than free ligand [26]. Such higher activity of the metal chelates can be explained on the basis of overtone's concept and chelation theory. Based on overtone's concept, the cell is surrounded by lipid membrane which allows the passage of lipid soluble material thus controls antibacterial activity. On the basis of chelation, the polarity of the metal ion will be reduced to a larger extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, the complexes become very stable due to the delocalization of p-electrons and enhance the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of pathogens.

These complexes also interrupt the respiration process of the cell and thus block the synthesis of proteins, which controls further growth of the microorganism [27].

In antifungal activity, the results showed that the mononuclear mixed ligand schiff bases metal complexes are more active against both fungi then the Schiff base. Co(II) complexes exhibited higher activity in both fungal strains than other complexes. This may result in to inhibitory in toxicology of metal ions towards some pathogens.







Fig. 8: Antifungal activity for of mixed ligand Schiff base complexes

3.7. DNA cleavage studies

Even after a longer exposure time, Control using DNA alone do not show any significant cleavage of pUC18DNA.The damage of DNA in Co(II), Ni(II) and Cu(II) complexes could be attributed to the DNA cleavage in the presence of oxidant which may be due to hydroxyl radical (OH'). The OH' free radicals participate in general oxidative mechanisms of deoxyribose moiety, followed by hydrolytic cleavage via abstraction of a hydrogen atom from sugar units. The results show that the important role of mixed ligand complexes in DNA cleavage reaction is shown in Fig.9. As the compound was perceived to cleave the DNA, it can be concluded that, all complexes effectively cleave the DNA as compared to control DNA and thus compound prevents the growth of pathogenic organism.



Fig. 9: The gel picture of DNA cleavage activity on pUC18DNA, Lane 1: DNA alone; Lane 2: DNA+ H_2O_2 ; Lane 3: DNA+ H_2O_2 +CuL¹L²; Lane 4: DNA+ H_2O_2 +CoL¹L²; Lane 5: DNA+ H_2O_2 +NiL¹L²

4. CONCLUSION

In the present work, Cu(II), Co(II) and Ni (II) complexes have been synthesized from Schiff bases (L^1, L^2) in 1:1:1 ratio and its non-electrolytic nature has been deduced from conductance data. The stoichiometry of complexes has been confirmed by elemental analyses, IR, Far-IR, 'H NMR, UV, Cyclic voltammetry, ESR spectral data and molar conductance studies. Anoctahedral geometry for all complexes has been assigned on the basis of electronic and magnetic moment. The antimicrobial activity results reveal that the cobalt complexes act as more bactericidal and fungicidal agents as compared to the un-complexed Schiff base ligands. The DNA cleavage properties of the mixed ligand complexes have been examined by gel electrophoresis method and results exhibited that all complexes efficiently undergoes DNA cleavage activity via hydroxyl radical pathway.

5. REFERENCES

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