Asian Journal of Chemistry; Vol. 32, No. 8 (2020), 1903-1908

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2020.22700

DNA Binding Potency and Antimicrobial Analysis of New Indole and Pyrazolone Based Transition Metal(II) Complexes

MURUGAN SELVAMARIAMMAL[1,*] and MARUTHAPPAN MALARVIZHI[2]

[1]Department of Chemistry, Sri S. Ramasamy Naidu Memorial College, Sattur-626203, India
[2]Research Department of Chemistry, The Madura College, Madurai-625011, India

*Corresponding author: E-mail: selvamariammal23384@gmail.com

Received: 28 February 2020; *Accepted*: 22 April 2020; *Published online*: 27 July 2020; AJC-19965

Some novel series of 5-chloro isatin and 4-aminoantipyrine based Schiff bases with transition metal(II) complexes of (Cu, Co, Ni and Zn) have been prepared and characterized by physical, analytical and spectral data. The synthesized ligand behaves as a neutral tridentate is confirmed by spectral techniques. During the complexation, the stoichiometry ratio 1:2 (metal:ligand) is followed and an octahedral arrangement is adopted by all the metal complexes. The calf-thymus DNA interacts with complexes *via* an intercalative mode is studied by electronic absorption titration. Moreover, all these synthesized metal(II) complexes were tested against a set of bacterial and fungal strains reveals that complexes exhibit better activity than free ligand.

Keywords: 5-Chloro isatin, 4-Aminoantipyrine, Antimicrobial studies, DNA binding.

INTRODUCTION

In the field of bioinorganic, the Schiff base complexes has been much more interest and it will provide the models for biologically important classes like antioxidant, antiproliferative, antimicrobial, anti-inflammatory *etc*. [1,2]. Generally, Schiff base form stable complexes with a number of metal ions because of their chelating nature and this leads to many applications [3,4]. Moreover, Schiff base ligand prepared by using the derivative of N-heterocyclic compounds show a wide range of biological activities and they have more number of possibilities of coordination to metal centers. Most of the medicinally important natural products possess indole group [5]. Recently, the cellularly active and the unique member in the family of Schiff base is isatin [1*H*-indole-2,3-dione] is an endogenous and it play a vital role in biological actions [6]. Also, it contains additional donor sites which make the compound become more versatile and flexible. This versality can produce a variety of complexes with transition and inner transition metals and it has attracted towards many researchers [7,8]. Further, another heterocyclic important compound like 4-aminoantipyrine (4-amino-2,3-dimethyl-1-phenyl-3pyrzolin-5-one) contain pyrazole ring. Its bioactivities include anticancer [9], analgesic [10], anti-inflammatory [11], antimicrobial [12]. DNA acts as a target molecule during the treatment of anticancer, with metal complexes it binds by covalent and non-covalent interactions [13-15]. In non-covalent interaction, DNA binds with complexes by intercalation or groove binding. Especially, planar aromatic ligand interacts with DNA by intercalation which is of interest to researchers [16]. This metallointercalator exhibit potential annihilation activity which is accompanied with their DNA binding capacity [17]. Thus, from the above consideration we have synthesized four complexes derived from the Schiff base ligand (Z)-5-chloro-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)imino)indolin-2-one were effectively explored to DNA binding and screened for antimicrobial activity by absorption and well diffusion methods, respectively.

EXPERIMENTAL

The chemicals and solvents were used as AnalaR grade. 5-Chloro isatin, 4-aminoantipyrine and calf-thymus DNA are supplied from Sigma-Aldrich and used as such. All the metal chlorides were obtained from Merck. The IR spectrum of ligand and its complexes were recorded using KBr pellets in FTIR 8400s spectrometer (4000-400 cm^{-1}). Electronic spectra were

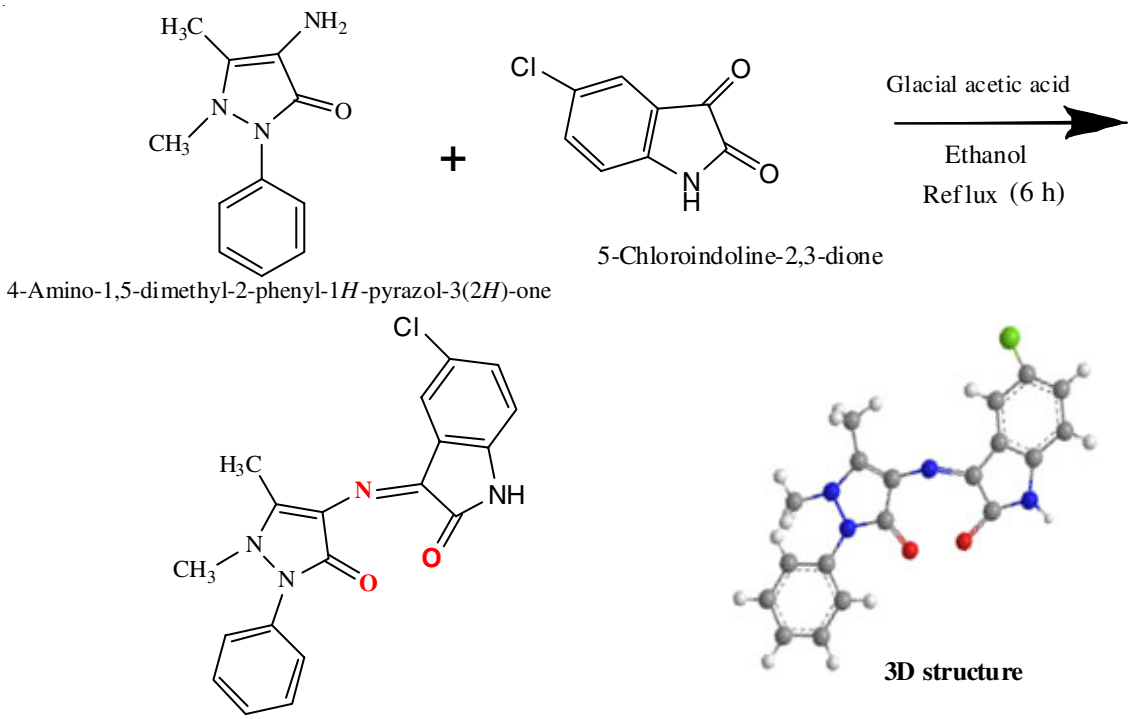
recorded between 200-800 nm wavelength ranges on a Shimadzu UV-spectrophotometer in DMSO solvent. At room temperature, the ^{1}H NMR spectra of the ligand and zinc complex were recorded in $CDCl_3$ on a BRUKER 300 MHz spectrometer using TMS as a standard. The studies of DNA binding were carried out by absorption technique using Tris-HCl buffer (pH = 7.2). The antimicrobial activity was performed by well diffusion method on both ligand and its metal complexes.

Synthesis of ligand (L): An ethanolic mixture of 5-chloro isatin (10 mmol) and 4-aminoantipyrine (10 mmol) was stirred in presence of acetic acid and refluxed for 6 h. The obtained orange precipitate of powdery mass was filtered and recrystallized using hot ethanol. It is dried over anhydrous calcium chloride (**Scheme-I**). Exact mass: 366.09; m.w.: 366.80; *m/z*: 366.09 (100.0%), 368.09 (32.7%), 367.09 (22.3%), 369.09 (6.7%), 368.10 (2.1%). Elemental analysis of $C_{19}H_{15}N_4O_2Cl$ (found): C, 62.21; H, 4.12; Cl, 9.67; N, 15.27; O, 8.72.

Synthesis of Schiff base metal complexes: An ethanolic solution of the synthesized Schiff base ligand (10 mmol) was appropriately mixed with corresponding metal(II) chloride salt in the 2:1 molar ratio. The stirring mixture was refluxed for 4 h. After the completion of the reaction, the resultant product was filtered and purified from ethanol and then dried *in vacuo* (**Scheme-II**).

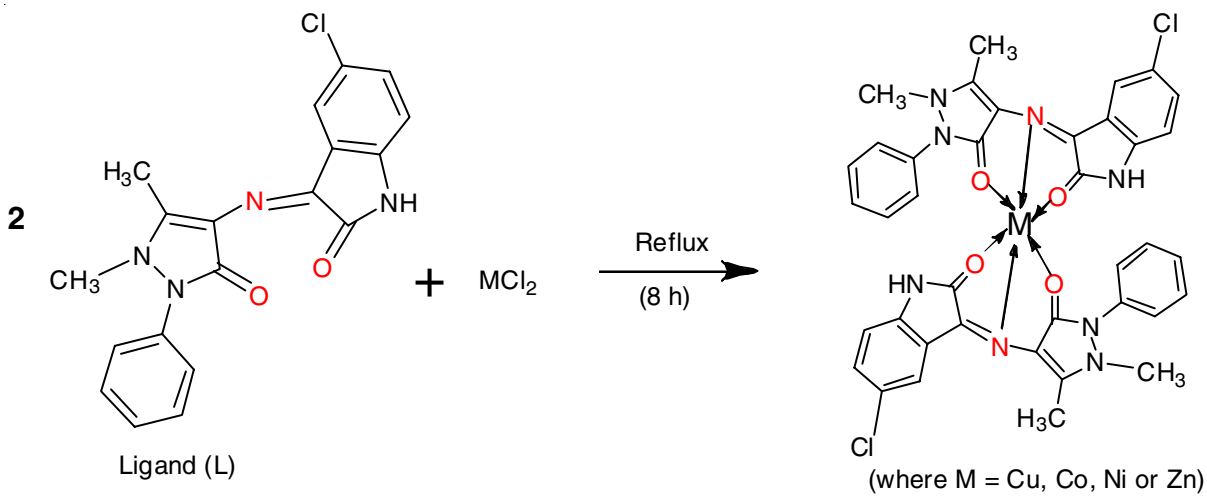
RESULTS AND DISCUSSION

Electronic spectra: Using DMSO as solvent, the absorption spectra of ligand and its complexes were recorded in the



(Z)-5-Chloro-3-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)imino)indolin-2-one

Scheme-I: Synthesis of Schiff base ligand (L)



Scheme-II: Synthesis of metal complexes $[ML_2]Cl_2$

range of 200-800 nm (Fig. 1). Two weak bands around at 284 and 318 nm showed by the ligand corresponding to π→π* and n→π* transitions, respectively. It is due to the aromatic π-electrons and the available non-bonded electrons of azomethine group. In complexation, there is a slightly changed of these characteristic bands indicate the coordination of ligand to the metal ion. An extra *d-d* transition bands are observed in the range of 655-671 nm in all the metal complexes which is absent in the ligand [18]. This transition is useful to predict the geometry of the complexes. The *d-d* transition assigned to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ responsible for cobalt and nickel complex respectively. These are all conclude that the complex exist in an octahedral geometry [19,20]. Zinc complex show only INCT band and it does not exhibit any *d-d* band because of its completely filled d^{10} configuration (Table-1).

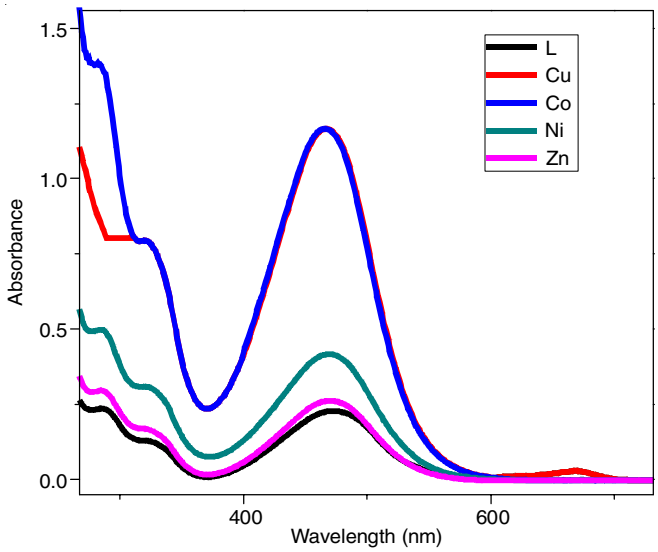


Fig. 1. Electronic spectra of ligand and its metal(II) complexes

TABLE-1
UV-VISIBLE SPECTRAL DATA AND
MAGNETIC MOMENTS OF THE COMPLEXES

Compound	Absorption (cm^{-1})	Assignments	μ_{eff} (BM)
Ligand	35211	π→π*	–
	31446	n→π *	
$[CuL_2]Cl_2$	35335	π→π *	1.76
	31055	n→π *	
	21505	LMCT	
	14903	$^2T_2g \rightarrow {}^2Eg$	
$[CoL_2]Cl_2$	35460	π→π *	4.56
	31250	n→π *	
	21186	LMCT	
	15015	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$	
$[NiL_2]Cl_2$	35335	π→π *	3.35
	31347	n→π *	
	21321	LMCT	
	15267	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$	
$[ZnL_2]Cl_2$	35087	π→π *	–
	31347	n→π *	
	21276	INCT	

Vibrational spectra: The IR spectral studies demonstrates that the binding of a functional group in a ligand to metal ion. By the comparison of both ligand and its metal complexes of vibrational spectra, the metal complex formation has been ascertained. The substituted isatin containing free ligand showed at 1732 cm^{-1} band is due to carbonyl group assignable to ν(C=O) vibration. The shifted lower frequency of this carbonyl group stretching in 1733-1722 cm^{-1} region in all metal complexes confirms the oxygen from carbonyl group is coordinated to the metal ion [21]. At 3147 cm^{-1} and 3048 cm^{-1} of two weak bands shows the stretching ν(N-H) from one compound and ν(C-H) of aromatic respectively [22-25]. In all the complexes, there is a new band appeared around at 560-550 cm^{-1} is a non-ligand band assigned for the substantiation of ν(M-O) bond [26]. The peak appears at 1625 cm^{-1} responds to the formation of azomethine nitrogen in the free ligand and the ligand coordination through this azomethine nitrogen is confirmed by the decrease in frequency of >C=N- in ligand to metal complexes. The presence of a new band around 510-490 cm^{-1} assignable to ν(M-N) is further substantiated this coordination (Fig. 2) [27]. From the above observation leads to the ligand coordinated to the metal ions by one nitrogen atom of azomethine group and oxygen atoms of the carbonyl group and it behaves as a neutral tridentate ligand (Table-2).

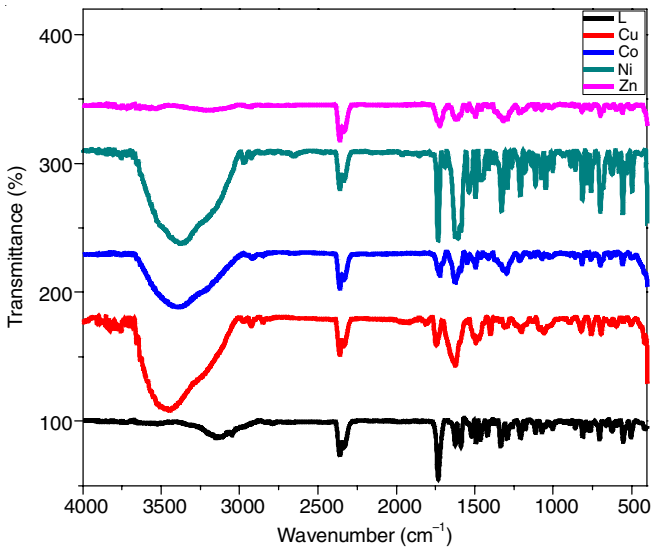


Fig. 2. IR spectra of ligand and its metal(II) complexes

^{1}H NMR spectra: The important information on the proton environment present in the compound is given by the ^{1}H NMR spectrum. From these spectra, the peak assignment for the proton resonances is calculated. This calculation is based on their peak multiplicity, intensity pattern and correlation of the integration values of the protons with the predicted pattern. Using tetramethyl silane as the internal standard, the proton NMR spectra of the ligand and its diamagnetic Zn(II) complex were recorded in $CDCl_3$. A singlet peak is observed at 8.39 ppm (S, 1H) corresponding to 5-chloro isatin NH proton of the ligand [28]. However, the evidence for the non-involvement of this NH of isatin is the retainment of its chemical shift value in the spectrum of Zn (II) complex. The protons in the phenyl

TABLE-2
IR SPECTRAL DATA (cm^{-1}) OF SCHIFF BASE LIGAND AND ITS METAL COMPLEXES

Compound	ν(N-H)	ν(C=O)	ν(C=N)	ν(C-Cl)	ν(M-O)	ν(M-N)
Ligand	3147	1732	1625	811	–	–
$[CuL_2]Cl_2$	3447	1744	1622	821	552	502
$[CoL_2]Cl_2$	3383	1723	1626	815	558	500
$[NiL_2]Cl_2$	3357	1733	1605	813	556	496
$[ZnL_2]Cl_2$	3199	1722	1611	816	559	502

ring are exhibited as the aromatic phenyl multiplets in the range of 6.75-7.48 ppm. The presence of the three hydrogen atoms in the form of C-CH_3 and N-CH_3 peaks at 2.49 and 3.34 ppm also displayed by the ligand spectrum [29]. When compared to the ligand a downfield region peak at 9.02 ppm is observed in the complex indicating that the coordination between the ligand azomethine group and the metal ion [30]. The ^{1}H NMR spectrum of both ligand and their zinc complexes having the corresponding phenyl, N-CH_3 and C-CH_3 peaks appeared in the same region.

DNA binding study by UV-visible absorption titration: In this study, using TrisHCl/NaCl buffer solution pH = 7.2, the binding inclination of metal(II) complexes were performed. The interaction of DNA with complexes were studied by increasing amount of CT-DNA (each addition of 30 µL) titrated progressively at a fixed concentration of the metal complexes. The charge transfer absorption bands of the complexes between 450-460 nm is monitored as the function of DNA. The studies reveals that there is a blue shift about 2-4 nm and a significant amount of 57-87% hypochromism is observed in all the complexes (Fig. 3). The intercalative binding strength is usually parallel to the extent of hypochromism. This consideration to be credited for the stacking interactions between the chelating ligand of the complexes with calf-thymus DNA *via* intercalative binding [31-34]. The experimental data were fit to the simple following Scatchard equation [35].

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b \varepsilon_f)}$$

where [DNA] is the concentration of the nucleic acid in base pairs, ε_a is the apparent absorption coefficient; ε_f and ε_b are the absorption coefficients for the free and the fully bound metal complexes respectively. Moreover, the absorption titrated spectrum is plotted against [DNA]/($\varepsilon_a - \varepsilon_f$) and [DNA].

The degree of interaction strength is measured by the binding constant (K_b). The ratio between the slope and the intercept give the value of K_b *i.e.*, hypochromism reveals that the conformational variation of nucleic acid helix in addition with change in environment polarity. The data of the binding parameters are given in Table-3. From these observations, it is specified that the synthesized complexes having more intercalating ability due to the presence of aromaticity, pyrazolone moiety and the electron withdrawing group which can improve the binding capacity. When compared to all metal complexes, nickel complex acts as a higher metallointercalator. Depends upon the size, ionic radius and charge on the metal ion the binding order varied which represent that the metal ion plays a vital role in DNA binding affinity [36].

TABLE-3
ELECTRONIC ABSORPTION SPECTRAL DATA FOR THE INTERACTION OF CT-DNA WITH SYNTHESIZED METAL COMPLEXES

Complex	λ_{max}		Δλ (nm)	[a]H (%)	$K_b \times 10^4$ (M^{-1})
	Free	Bound			
$[CuL_2]Cl_2$	457	454	3	57	2.5
$[CoL_2]Cl_2$	456	452	4	75	1.2
$[NiL_2]Cl_2$	459	457	2	85	5.3
$[ZnL_2]Cl_2$	458	456	2	81	4.2

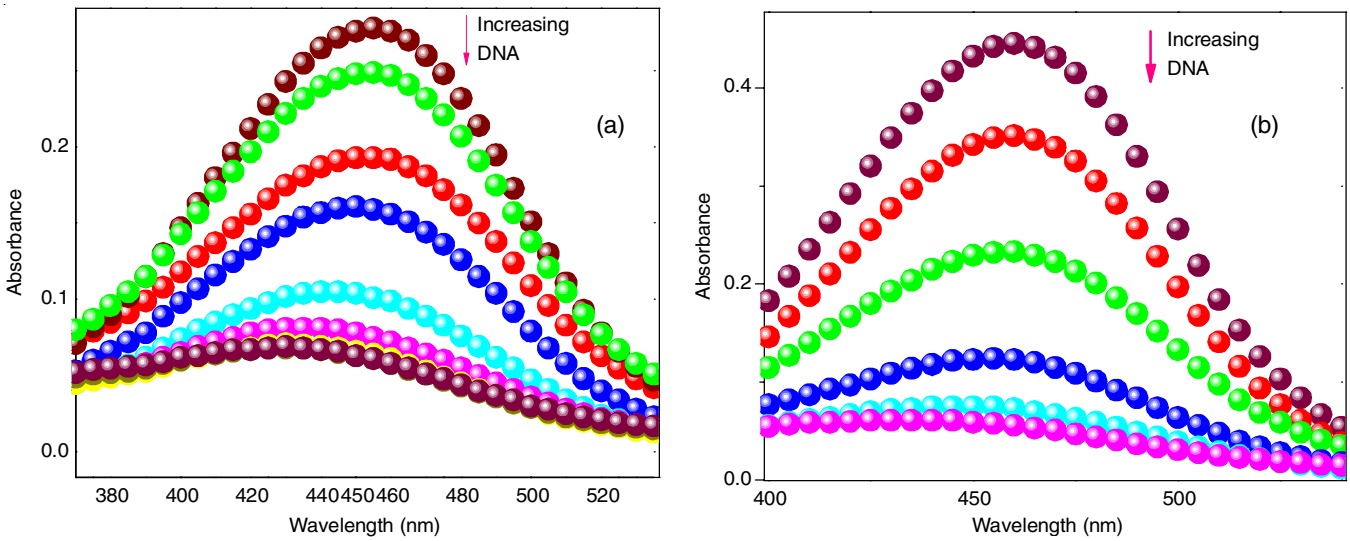


Fig. 3. Increasing amount of DNA (a) cobalt (b) nickel complexes in Tris-HCl buffer [electronic absorption]

TABLE-4
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY DATA OF SYNTHESIZED SCHIFF BASE LIGAND AND ITS COMPLEXES

Test organisms	Zone of inhibition (mm)						
	L	Cu	Co	Ni	Zn	Standard amikacin/ clotrimazole	Solvent control
Pseudomonas aeruginosa	12	15	26	19	18	23	NZ
Bacillus subtilis	9	14	16	17	15	28	NZ
Aspergillus niger	9	11	12	10	15	18	NZ

***in vitro* Studies of antimicrobial screening effects:** Antimicrobial activity was carried out using well diffusion method. The bacterial strains were inoculated in nutrient broth and incubated for 24 h before use in antibacterial assay. Sterile Muller-Hinton agar (Hi-media) plates were prepared and allowed to set. Three wells were cut onto the solidified agar plate using cork borer. The cultures to be screened were swabbed on top of the solidified media. The given samples were dissolved in dimethyl sulfoxide (DMSO) and 50 µL of sample was poured in the well. DMSO was used as the control. Amikacin (antibiotic) was used as standard. The plates were incubated in sterilization chamber itself for 30 min to allow the extract to diffuse into the medium. At 37 °C the plates were incubated for 24 h in inverted position. After incubation, the inhibition zone was measured in millimeter (mm). Assay was carried out in triplicates and the mean values were reported. Through biochemical and morphological modifications, the bacterial strains can achieve resistance to antibiotics [37]. The antimicrobial activity of the synthesized compounds were tested against selected Gram-positive bacterial strains like *Bacillus subtilis* and Gram-negative bacterial strains such as *Pseudomonas aeruginosa* and the fungal strain namely *Aspergillus niger*. Moreover, the antimicrobial activities of the synthesized compound were also compared with the standard amikacin (antibacterial agent) and clotrimazole (antibiotic). A comparative study of the MIC (minimum inhibitory concentration) values of Schiff base and its metal complexes are given in Table-4. The data show that in microorganism the inhibitory activity of production of enzyme is enhanced by the coordination of metal ion with the ligand containing nitrogen and oxygen atoms [38]. On the basis of Overtone's concept and Tweedy's theory, the enhancement of antimicrobial activity of the complexes can be explained [39]. Upon chelation, the overlapping of ligand orbital with the metal orbitals leads to reduction in the polarity of the metal ion and thereby resulting there is a metal positive charge partially shared with the donor atoms of the ligand. Therefore, we observed over the whole chelate ring having a delocalization of positive charge. The penetration of the bacterial cell membrane was enhanced by the increased lipophilic nature of the metal complexes and also the binding sites of metal on enzymes will be blocked. Therefore, the normal metabolic pathways of these microorganisms is hindered by the interaction between the lipid and the metal ion. According to Overtone's concept, the passage of only lipid soluble materials favours by the cell due to an important factor of lipo-solubility that controls the antimicrobial activity. The bulkiness of the synthesized compounds is directly proportional to the antimicrobial activity [40,41]. Further microorganism growth was controlled by these metal complexes due to the blocking of the protein synthesis and agitates the respiration process of the cell [42].

Conclusion

A series of transition metal(II) complexes with 5-chloro isatin and pyrazolone based moiety were synthesized. The formation of compounds was ensured by the various spectral techniques like electronic, vibrational, proton nuclear magnetic resonance and the analytical studies. The exhibited octahedral geometry of the metal complexes is based on its physico-chemical observation. The interaction of all these metal complexes with CT-DNA credibly by the way of intercalation binding mode which is explored by the electronic absorption titration. Compared to other metal complexes, nickel complex having remarkable binding affinity is symbolized by its K_b values. The ligand and its metal complexes have improved antimicrobial activity which is proved by the analysis against a set of microbial strains and seductively. The cobalt complex exhibited potential activity compared to other metal complexes. Overall, a significant effect is observed in both biological properties as well as interaction with DNA by the presence of the metal ion, functional group on the main ligand, indole and pyrazolone moieties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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