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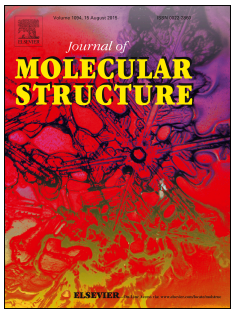

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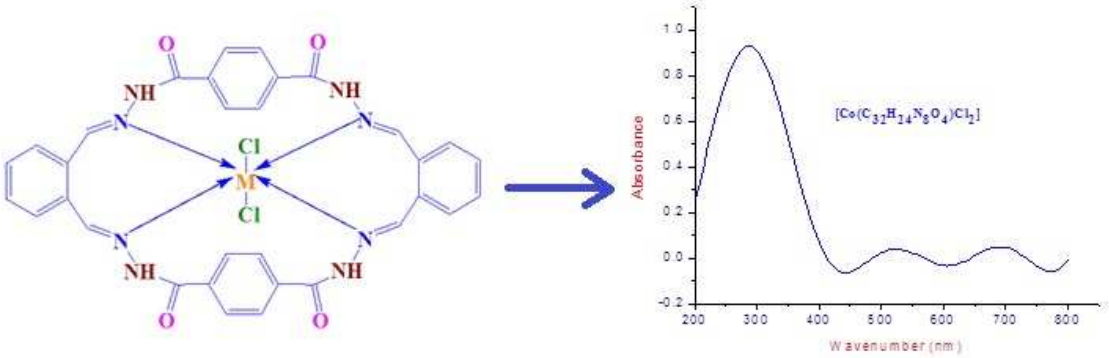

Design, synthesis and characterization of macrocyclic ligand based transition metal complexes of Ni(II), Cu(II) and Co(II) with their antimicrobial and antioxidant evaluation.

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Abstract

Three new complexes Ni(II), Cu(II) and Co(II) were synthesized of macrocyclic ligand derived from 1, 4-dicarbonyl-phenyl-dihydrazide and *O*-phthalaldehyde in the ratio of 2:2. The synthesized compounds were characterized by elemental analyses, molar conductance, magnetic susceptibility measurements, FTIR, UV–Vis., Mass and ^{1}H NMR spectral studies. The electronic spectra of the metal complexes indicate a six coordinate octahedral geometry of the central metal ion. These metal complexes and the ligand were evaluated for antimicrobial activity against bacteria (*E. coli, B. subtilis, S. aureus*) and fungi (*A. niger, A. flavus, C. albicans*) and compared against standard drugs chloramphenicol and nystatin respectively. In addition, the antioxidant activity of the compounds was also investigated through scavenging effect on DPPH radicals.

Keywords: Metal complexes, Macrocycle, ligand, Spectroscopic study, Antimicrobial activity, Antioxidant activity

1. Introduction

In the last two decades coordination chemistry of macrocyclic ligands have shown a considerable interest [1, 2]. The use of macrocyclic ligands as models for protein-metal binding sites in biological systems, such as the synthetic ionophores, models for the magnetic exchange phenomena, therapeutic reagents in chelate therapy for treatment of metal intoxication and the cyclic antibiotics that retain their antibiotic actions to specific metal complexation [3–6] raises the importance of new macrocyclic ligand designing. The great importance of macrocyclic systems in chemistry is due to their selective chelation towards certain metal ions depending on the number, type and position of their donor atoms, the ionic radii of the metal centers, and the coordinating property of the counter ions [7]. The synthetic macrocyclic complexes mimic the naturally occurring bio-macrocyclic systems such as iron-porphyrin core in haemoglobin, cobalt-corrin of vitamin B_{12} and magnesium-hydroporphyrin

in chlorophyll [8] which boost their importance from the biological point of view. There are a number of important macrocyclic molecules which show biological activities including antibacterial, antifungal, antidiabetic, antiproliferative, anticancer, herbicidal and anti-inflammatory activities [9-16].

Microbial diseases have become a threat even in the current century being still very much problematic to diagnose. During the last century various types of antibacterial and antifungal drugs have been discovered [17-19]. Presently antibacterial sulfa drugs, nitrofuranes, penicillins, cephalosporins, tetracyclines and oxaolidin and antifungal agents such as fluconazole, ketoconazole and amphotericin B are used as antimicrobial agents [20-23]. There are still various problems unsolved for various antimicrobial agents present till date. Fungal infections are not only limited to superficial tissues but are also reported for the life threatening level [24]. The main reason for this serious level may be owing to the huge number of patients at risk, including those with progressive age, major surgery, immunosuppressive therapy, acquired immunodeficiency syndrome (AIDS), cancer treatment and solid-organ and hematopoietic stem cell transplantation [25]. To develop the more effective antimicrobial drugs is the need of an hour and the macrocyclic metal complexes are also known to act as promising antimicrobial agents [26-29].

In this manuscript, the synthesis, spectral characterization and biological properties of the new Ni (II), Cu (II) and Co (II) complexes of 1, 4-dicarbonyl-phenyl-dihydrazide with *O*-phthalaldehyde have been described to shed more light on the structure and biological properties of this new series of metal complexes.

2. Experimental

2.1. Reagents

All chemicals used were of the analytical grade. Diethyl terephthalate, *O*-phthalaldehyde, and hydrazinhydrate were procured from Aldrich. Metal salts were purchased from Merck.

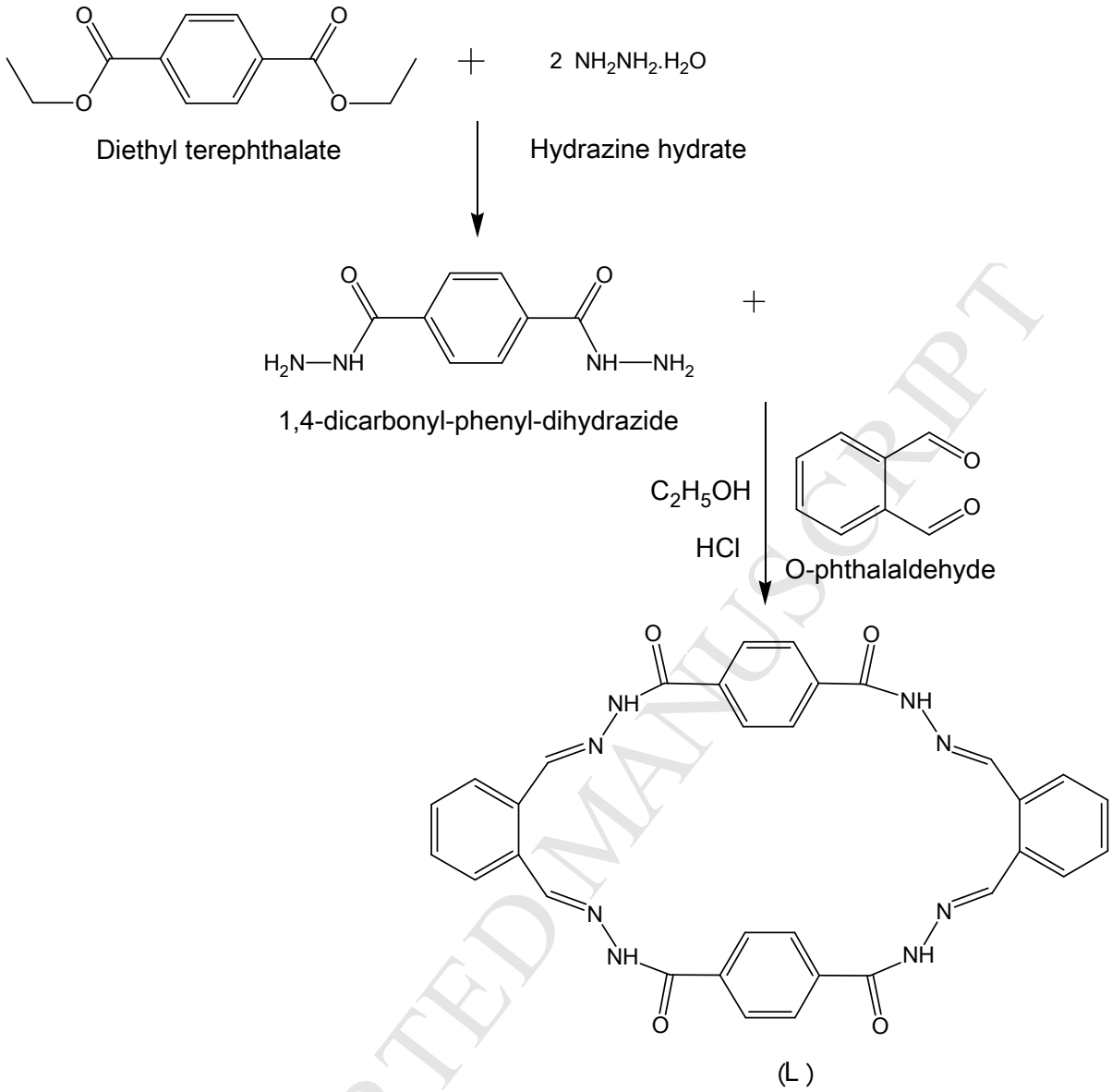
2.2. Synthesis of 1, 4-dicarbonyl-phenyl-dihydrazide

Diethyl terephthalate (2.22 g) was dissolved in 30 mL mixture of ethanol. The solution of diethyl terephthalate was added to a solution of hydrazine hydrate (98% 2 mL) dissolved in 20 mL ethanol. After complete addition, the solution was stirred for 4 h. After stirring, the solvent was removed by rotary evaporation under reduced pressure. The resulting dihydrazide of terephthalic acid was collected, washed with water and then recrystallized from methanol and dried in vacuum [30].

2.3. Synthesis of macrocyclic ligand

A solution of 0.27 g of *O*-phthalaldehyde (2 mmol) in 25 ml ethanol was reacted with 0.39 g (2 mmol) of 1,4-dicarbonyl-phenyl-dihydrazide in 30 ml ethanol in a round bottom flask. Stirring was continued for 3-4 h at room temperature during which a solid compound separated. It was filtered, washed with methanol, recrystallized from dichloromethane/methanol and dried in vacuum. (Scheme 1)

Melting point 145ºC and Yield 65-70% ^{1}H NMR (400 MHz, DMSO-d_6) δ=7.30-7.80 (m, 16H, Ar–H), 7.98 (s, Ar-H, carbonyl Phenyl), 9.15 (s, 4x1H, -NH of hydrazide). ^{13}C NMR (400 MHz, DMSO-d_6) δ = 137.2 (C═N), 170.5 (C═O), 138.5, 135.2, 130.1, 128.9, 127.4 (Ar–C). UV/vis (1 x 10^{-4} mol, DMSO): λ (cm^{-1}) = 27777, 25641, 22471. IR (KBr disc, cm^{-1}) ν(N–H) 3250, ν(C=N) 1625, ν(C=O) 1720, ν(N–N) 1605, ν(C–C) 1105, ν(C=C, aromatic) 755, ν(C–H, aromatic) 1536.



Scheme 1. Synthesis of Macrocyclic ligand.

2.4. Synthesis of the Ni(II), Cu(II) and Co(II) complexes

The metal complexes were synthesised by refluxing a methanolic solution of macrocyclic ligand (1.0 mmol) and $M(Cl)_2$ metal salts (1.0 mmol) for 2 h during which a precipitate separated. The precipitate was filtered off, washed with ethanol and dried under vacuum over anhydrous $CaCl_2$ (65-72% yield) (Scheme 2).

2.4.1. Synthesis of metal complex (1)

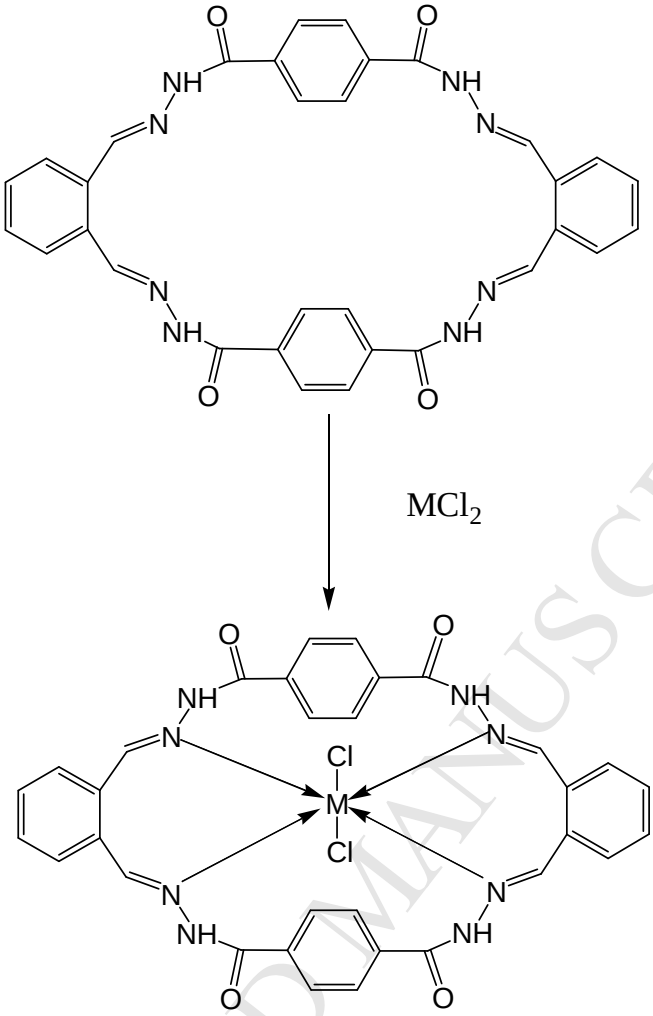
Synthesis of $[Ni(C_{32}H_{24}N_8O_4)Cl_2]$ complex(1). A solution of $NiCl_2.6H_2O$ in methanol (15 mL) was mixed with the hot solution of the macrocyclic ligand (L) in ethanol (1:1) and the reaction was refluxed for 6 hours till the precipitate appeared. The precipitate was filtered off, washed with methanol and dried under vacuum over anhydrous $CaCl_2$ Yield 72%. UV/vis (1 x 10^{-4} mol, DMSO): λ = 21097 ($^3A_{2g}$ (F) → $^3T_{1g}$ (P)), 13793($^3A_{2g}$ (F) → $^3T_{1g}$ (F)), 12254($^3A_{2g}$ (F) → $^3T_{2g}$ (F)). IR (KBr disc, cm^{-1}) ν(N–H) 3235, ν(C=N) 1635, ν(C=O) 1720, ν(N–N) 1612, ν(C–C) 1105, ν(C=C, aromatic) 755, ν(C–H, aromatic) 1536, ν(M–N) 434, ν(M–Cl) 316.

2.4.2. Synthesis of metal complex (2)

Synthesis of $[Cu(C_{32}H_{24}N_8O_4)Cl_2]$ complex (2). A solution of $CuCl_2.2H_2O$ in methanol (15mL) was mixed with the hot solution of the macrocyclic ligand (L) in ethanol (1:1) and the reaction was refluxed for 6 hours till the precipitate appeared. The precipitate was filtered off, washed with methanol and dried under vacuum over anhydrous $CaCl_2$ Yield 68%. UV/vis (1 x 10^{-4} mol, DMSO): λ = 15797 (^2E_g→$^2T_{2g}$). IR (KBr disc, cm^{-1}) ν(N–H) 3230, ν(C=N) 1640, ν(C=O) 1720, ν(N–N) 1612, ν(C–C) 1105, ν(C=C, aromatic) 755, ν(C–H, aromatic) 1536, ν(M–N) 450, ν(M–Cl) 310.

2.4.3. Synthesis of metal complex (3)

Synthesis of $[Co(C_{32}H_{24}N_8O_4)Cl_2]$ complex (2). A solution of $CoCl_2.6H_2O$ in methanol (15mL) was mixed with the hot solution of the macrocyclic ligand (L) in ethanol (1:1) and the reaction was refluxed for 6 hours till the precipitate appeared. The precipitate was filtered off, washed with methanol and dried under vacuum over anhydrous $CaCl_2$ Yield 73%. UV/vis (1 x 10^{-4} mol, DMSO): λ = 19047 ($^4T_{1g}$ (F) →$^4A_{2g}$ (F)), 14450 ($^4T_{1g}$ (F) →$^4T_{1g}$ (P)) IR (KBr disc, cm^{-1}) ν(N–H) 3225, ν(C=N) 1630, ν(C=O) 1720, ν(N–N) 1612, ν(C–C) 1109, ν(C=C, aromatic) 757, ν(C–H, aromatic) 1535, ν(M–N) 440, ν(M–Cl) 318.



Scheme 2. Synthesis of Macrocyclic metal complexes. (M = Ni, Co, Cu)

3. Pharmacology

3.1. Antimicrobial activity

The antimicrobial activities of the synthesised compounds (macrocyclic ligand and its metal complexes) have been screened in vitro, as growth inhibiting agents. The antifungal and antibacterial screening were carried out using Food Poison and Disc Diffusion Method against some strains of bacteria and fungi like *E. coli*, *B. subtilis,* and *S. aureus* and *A. niger*, *A. flavus* and *C. albicans* respectively. The compounds were dissolved in DMSO (1%) to get the required test solutions. The PDA and nutrient agar were used as a required medium for these activities. After incubation for 24 h at 27°C in the case of bacteria and for 7 days at

27°C in the case of fungi, the inhibition of the organisms evidenced by clear zone surround each disk was measured.

3.2. Antioxidant activity

The antioxidant activity of the compounds is evaluated by using the scavenging ability of the stable DPPH (2, 2′-diphenyl-1-picryl hydrazyl) radical. The scavenging capacity of $DPPH^{\bullet}$ was determined spectrophotometrically.

4. Results and discussion

The general composition for all the complexes was found to be MLX_2 (where M = Ni, Cu and Co; L = $C_{32}H_{24}N_8O_4$ and X=Cl^-). All the complexes provide proper C, H and N results which were in good agreement with those calculated for the suggested formulae. The results of elemental analyses with molecular formulae were listed in Table 1. The complexes obtained were variously colored, polycrystalline, air stable and insoluble in common organic solvents but readily soluble in DMSO and DMF.

4.1. Molar conductance measurements

The molar conductivities 10^{-3} M solutions of the synthesised metal Complexes in DMF were studied at room temperature. The complexes showed molar conductance values in the range of 12–18 $\Omega^{-1}cm^2mol^{-1}$ showing that complexes were non-electrolytic in nature [31,32]. The molar conductance value suggested that the complexes may be formulated as $[MLX_2]$ and the anions were inside the coordination sphere and bonded to the central metal ion.

4.2. Magnetic susceptibility measurements

The Ni(II) complexes showed magnetic moment value of 3.3 B.M. corresponding to the range normally observed for octahedral geometry [33]. The Cu(II) complexes showed magnetic moment in the range of 1.92–1.99 B.M., corresponding to one electron that falls within the range normally observed for six coordinated Cu(II) complexes [34]. The magnetic moment values of the Co(II) complexes were reported in the range of 4.72–4.95 B.M. which

is close to the normal range for octahedral complexes is (4.3– 5.2 B.M.), hence indicative of octahedral geometry [35,36]. The experimental values were higher than the spin only value due to orbital angular momentum contribution in d^7 system.

Table 1. Analytical data of the Cu(II), Co(II), and Ni(II) complexes.

Complexes	**Mol. wt.**	**Elemental analysis found (calculated) (%)**				μ_{eff}
		C	**H**	**N**	**M**	
$C_{32}H_{24}N_8O_4$ (L)	584.58	65.75 (65.69)	4.14(4.08)	19.17(19.11)	-	
$[Ni(C_{32}H_{24}N_8O_4)Cl_2]$	714.18	53.82 (53.77)	3.39(3.30)	15.69(15.58)	8.22 (8.18)	3.3
$[Cu(C_{32}H_{24}N_8O_4)Cl_2]$	719.04	53.45 (53.38)	3.36(3.28)	15.58(15.49)	8.84 (8.76)	1.92-1.99
$[Co(C_{32}H_{24}N_8O_4)Cl_2]$	714.42	53.80 (53.75)	3.39(3.31)	15.68(15.59)	8.96 (8.91)	4.72-4.95

4.3. IR spectra

The infrared spectrum which affords valuable evidence about the coordination of the synthesised compounds with the metal salts has been presented in Figure S1 (Supplementary Data). The absence of bands ~Ca. 3400 cm^{-1} characteristic of the free $–NH_2$ group supported the formations of macrocyclic ligand by the condensation between 1, 4-dicarbonyl-phenyl-dihydrazide and *O*-phthalaldehyde molecules. The occurrence of a medium intensity band at ca. 1550–1640 cm^{-1} attributable to the imine υ(C=N) stretching frequency [37,38], which appeared at lower frequency values in the complexes, which indicates that the coordination has took place through the C=N group. The appearance of a band at ca. 430–470 cm^{-1} in the spectra of the complexes, which was assigned to υ(M–N) is also an indication of the metal azomethine nitrogen bond. A medium intensity band at 3310–3250-3225 cm^{-1} was assigned to υ(N–H) stretching mode of –NHCO– moiety [39]. In the far IR region, the υ(M–Cl) and υ(M–N) bands appeared in the 310–330 and 420–445 cm^{-1} [40,41].

4.4. Mass spectra

Mass spectra provided additional structural information about the analysed species. The ESI mass spectra of the synthesised ligand $C_{32}H_{24}N_8O_4$ (L) exhibited the molecular ion peak at m/z 584.13 as shown in figure S2. The electronic impact mass spectrum of the Co(II) choro complex showed a molecular ion $[Co(C_{32}H_{24}N_8O_4)Cl_2]^+$ peak at m/z 715.06 amu (figure S3) and the molecular ion peak may be corresponding to isotopic peak (M+1) due to ^{13}C and ^{15}N isotopes. Cu(II) complex showed molecular ion $[Cu(C_{32}H_{24}N_8O_4)Cl_2]^+$ peak at m/z 720 amu and the Ni(II) complex at 715.18 which corresponds to the molecular ion peak of $[Ni(C_{32}H_{24}N_8O_4)Cl_2]^+$. In addition to the peaks due to the molecular ion, the spectra exhibit peaks assignable to various fragments arising from the thermal cleavage of the complexes [42]. The intensity of these peaks gave the idea regarding the stability and abundance of the ions. This data is in good agreement with the proposed molecular formula for these complexes.

4.5. ^1H NMR

The ^1H NMR spectra of macrocyclic ligand (L) (Figure S4) was recorded in dimethylsulfoxide (DMSO d_6) solution using Me_4Si (TMS) as internal standard. The ^1H NMR spectra of the ligand shows broad signal at 9.15 ppm due to the –NH [43,38], 7.98 ppm due to the –CH=N [44]. The multiplets in the region 7.30-7.80 ppm may be assigned to aromatic proton [45]. ^{13}C NMR spectra of the macrocyclic ligand and its metal complexes are recorded in dimethylsulfoxide (DMSO-d_6) solution, using tetramethylsilane (TMS) as internal standard. ^{13}C NMR spectrum of the symmetrical macrocyclic ligand displayed characteristic signals at δ 138.5 ppm, 135.2 ppm, 130.1 ppm, 128.9 ppm, 127.4 ppm corresponding to the aromatic carbons. The signal at 137.2 ppm and 170.5 ppm are due to C═N, and C═O respectively [46]. The ^{13}C NMR spectra of complexes show no appreciable changes compared with free ligand.

4.6. Electronic spectra

The electronic spectra of complexes were recorded in DMSO (Table 2). The spectrum of Ni(II) complex in DMSO exhibits three bands at 21,097, 13,793 and 12,254 cm^{-1} assigned to $^3A_{2g}(F)\rightarrow{}^3T_{1g}(P)$, $^3A_{2g}(F)\rightarrow{}^3T_{1g}(F)$ and $^3A_{2g}(F)\rightarrow{}^3T_{2g}(F)$ transitions, respectively, in an octahedral geometry around Ni(II) ion [47]. The spectrum shows also, a band at 33,003 cm^{-1} assigned to L→M charge-transfer.

The electronic spectrum of the macrocyclic copper complex shows broad band absorption in the range 15,797 cm^{-1}, which may be assign to $^2E_g \rightarrow {}^2T_{2g}$ transition and it is in conformity with octahedral geometry [48,49], an indication of the most probable geometric configuration of the synthesized metal complexes is their magnetic moment values. (Figure 1) The Co(II) complexes shows electronic spectral bands at 19047 cm^{-1} and 14450 cm^{-1} which were assigned to the transitions $^4T_{1g}$ (F) $\rightarrow{}^4A_{2g}$ (F) and $^4T_{1g}$ (F) $\rightarrow{}^4T_{1g}$ (P) respectively. The positions of bands indicated that Co(II) complexes have an overall octahedral geometry [50]. (Figure 2)

Table 2. Spectral absorption bands of synthesised macrocyclic metal complexes.

Compound	Band position (cm^{-1})	Assignment	Geometry
$[Ni(C_{32}H_{24}N_8O_4)Cl_2]$	21097	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$	Octahedral
	13793	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$	
	12254	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$	
$[Cu(C_{32}H_{24}N_8O_4)Cl_2]$	15797	$^2E_g \rightarrow {}^2T_{2g}$	Octahedral
$[Co(C_{32}H_{24}N_8O_4)Cl_2]$	19047	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	Octahedral
	14450	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$	

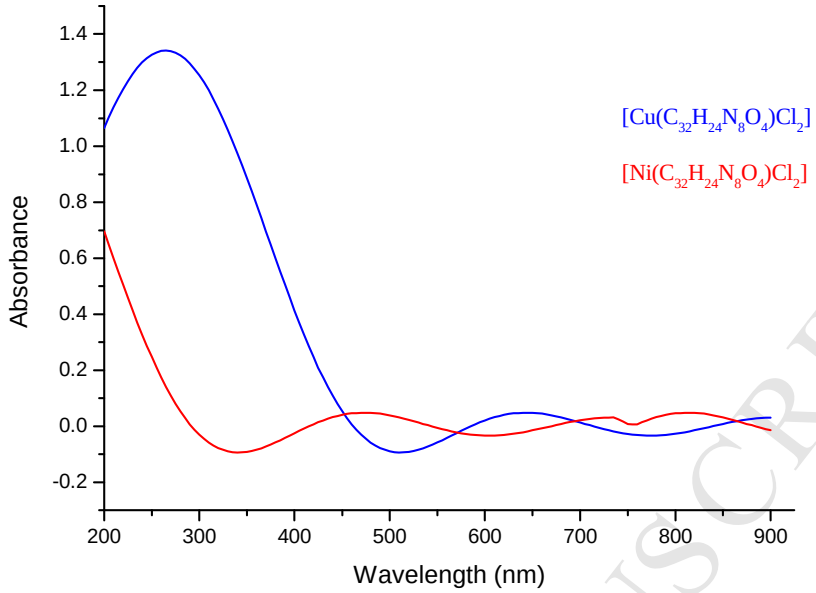


Figure 1. Electronic spectra of Nickle and Copper metal complexes

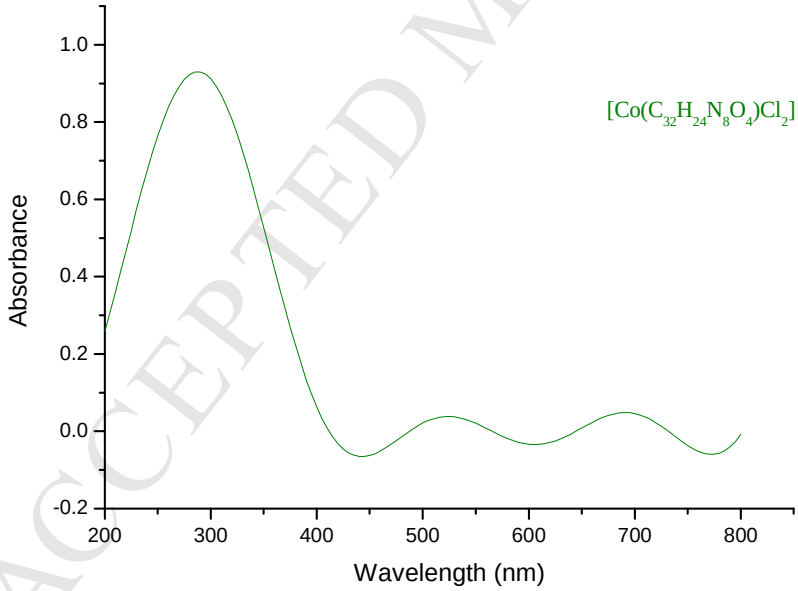


Figure 2. Electronic spectra of Cobalt metal complexes

4.7 X-ray diffraction analysis

The power X-ray diffraction patterns of the synthesised macrocyclic complexes were used for the cell refinement and pattern indexing of unit cells. The diffractogram reveals the

crystalline nature of the metal complexes and are worked out by using trial and error method for finding the best fit between observed and calculated values [51]. The powder XRD analysis of the macrocyclic complexes was recorded in the 10–80º (2θ) range and the results obtained show well resolved sharp peaks with high intensities. The observed sharp peaks indicate high degree of crystallinity for the complexes (Figure 3-5). The indexing, calculations of unit cell parameters, scattering angles (2θ) corresponding to each reflection and inter-planar spacing (d) along with Miller's indices were evaluated (Table S1-S3) which corresponds to triclinic, tetragonal, and monoclinic crystal system for Ni(II), Co(II) and Cu(II) complexes respectively.

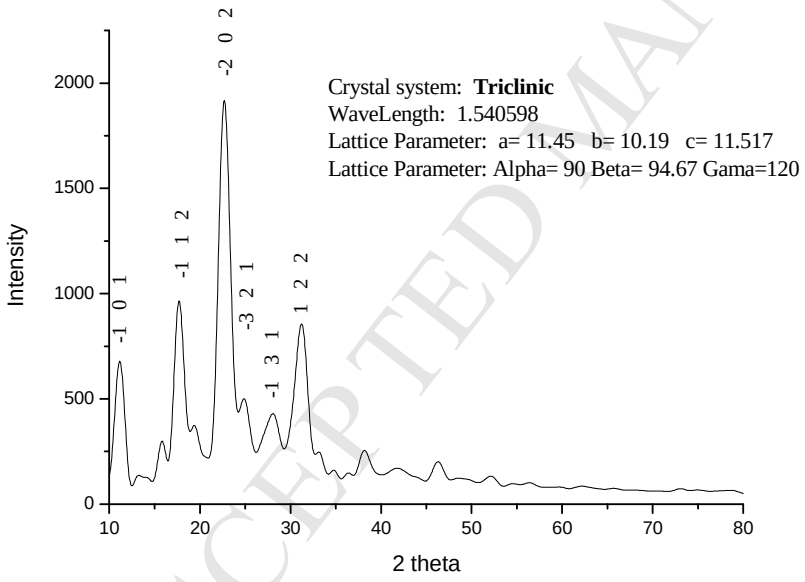


Figure 3. X-Ray diffractogram of $[Ni(C_{32}H_{24}N_8O_4)Cl_2]$

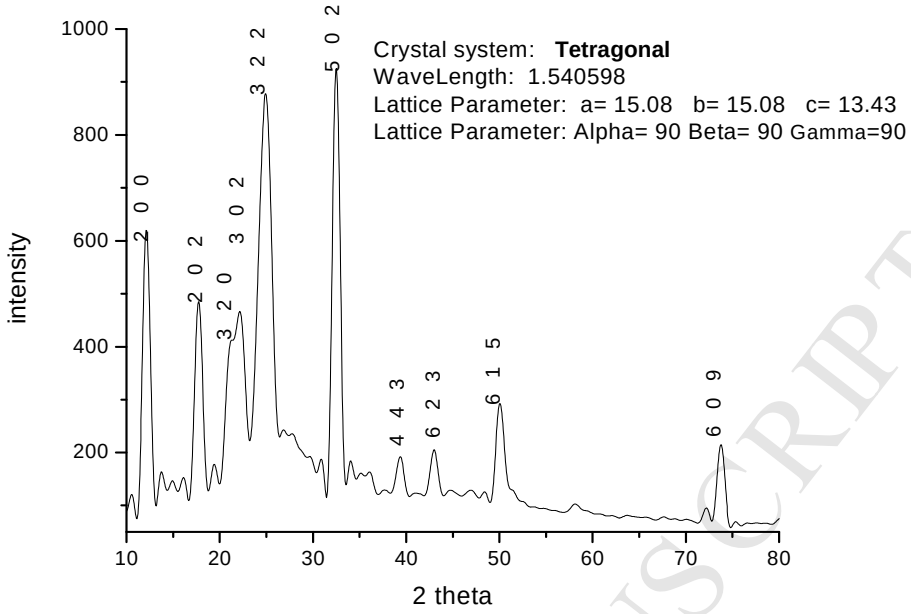


Figure 4. X-Ray diffractogram of $[Co(C_{32}H_{24}N_8O_4)Cl_2]$

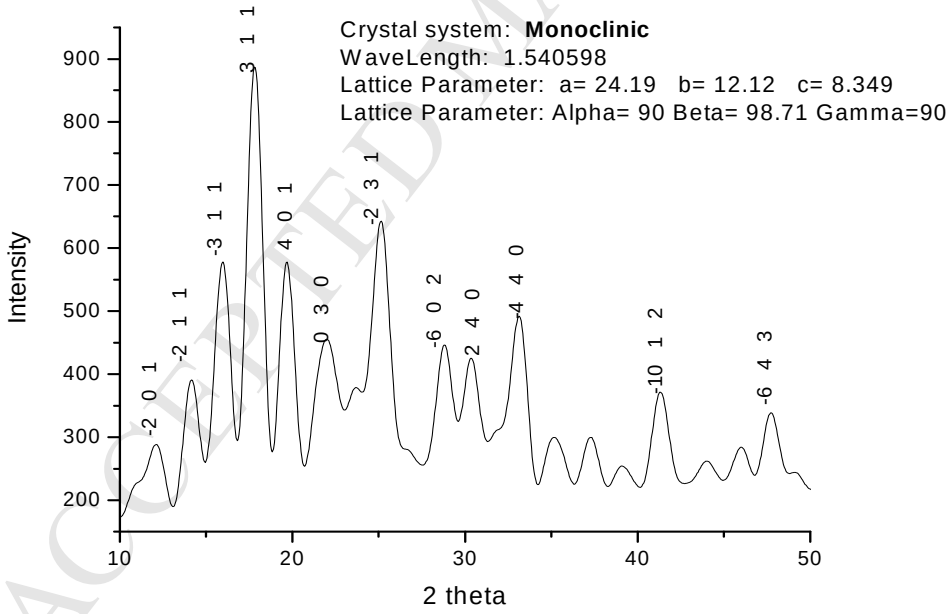


Figure 5. X-Ray diffractogram of $[Cu(C_{32}H_{24}N_8O_4)Cl_2]$

4.8. Antibacterial Activity

The antibacterial activity (Table 3) of the macrocyclic ligand and its complexes were determined by Disc Diffusion Method [52,53]. The test solutions were prepared by dissolving

the synthesised compounds in DMSO. 20 mL of the nutrient agar medium was poured on each petriplates and after it 0.5 mL of test bacterial broth was spread over the medium. From the test solution 25 μL was applied on the sterile disc having a diameter of 10 mm. after the solvent evaporation, the discs were placed at the centre of the inoculated petriplates and sealed with para film. The petriplates were incubated at 27°C for 24 h and the inhibition zones were measured in millimetres.

Table 3. Antibacterial activities of ligand (L) and its complexes.

Compounds	**Bacterial inhibition zone, mm (conc. in μgml^{-1})**								
	E. coli			*B. subtilis*			*S. aureus*		
	100	**250**	**500**	**100**	**250**	**500**	**100**	**250**	**500**
$C_{32}H_{24}N_8O_4$ (L)	-	-	09	-	06	08	-	-	09
$[Ni(C_{32}H_{24}N_8O_4)Cl_2]$	06	11	16	08	15	19	-	09	13
$[Cu(C_{32}H_{24}N_8O_4)Cl_2]$	10	14	18	05	12	18	07	13	16
$[Co(C_{32}H_{24}N_8O_4)Cl_2]$	07	09	12	07	11	16	08	11	14
Chloramphenicol	32	32	32	30	30	30	35	35	35

4.9. Antifungal Activity

The fungicidal activity (Table 4) of the synthesised macrocyclic ligand and its metal complexes was determined in vitro by Food Poison Technique [52, 54]. The required test solutions were prepared by dissolving the compounds in the DMSO. The required quantity of this solution was added to PDA medium and poured into the petriplate and the 5 mm mycelial disc of fungus was placed on the medium. Finally, the petriplates were sealed by the para film and were kept in the incubator at 27°C for 7 days. The activities were compared with the activity of the standard fungicide Nystatin. The inhibition of fungal growth expressed in percentage terms was determined from the growth in the test plates compared to the respective control plates as given below.

$$\text{Inhibition } \% = \frac{(\text{C} - \text{T})}{\text{C}} \times 100$$

Where, C is the Diameter of fungal growth in the control plate and T the Diameter of fungal growth in the test plate.

Table 4. Antifungal activities of ligand (L) and its complexes.

Compounds	Fungal inhibition, % (conc. in μgml^{-1})								
	A. niger			*A. flavus*			*C. albicans*		
	50	**100**	**150**	**50**	**100**	**150**	**50**	**100**	**150**
$C_{32}H_{24}N_8O_4$ (L)	17	28	32	15	30	39	12	18	31
$[Ni(C_{32}H_{24}N_8O_4)Cl_2]$	31	41	59	28	39	45	19	33	43
$[Cu(C_{32}H_{24}N_8O_4)Cl_2]$	26	49	51	21	40	50	24	37	51
$[Co(C_{32}H_{24}N_8O_4)Cl_2]$	29	39	52	29	46	57	13	21	35
Nystatin	69	84	92	70	89	94	52	68	81

From the results of the antibacterial and antifungal activity of metal complexes, it is obvious that the metal complexes exhibit greater antimicrobial activity than that of the free macrocyclic ligand; this progressive antibacterial activity of the metal complexes, compared with that of Schiff bases, is conceivably owing to modification in structure due to coordination, and chelating tends to make metal complexes act as more powerful bacteriostatic agents, thus inhibiting the growth of the microorganisms. The Overtone's concept and chelation theory explains the increased antimicrobial effect as the chelation have a tendency to make the ligand a more powerful and potent bacterial agent [55]. On chelation, the polarity of the metal ion will be reduced to a better range due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of p-electrons over the whole chelate ring and enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes may also disturb the respiration process of

the cell and thus block the synthesis of proteins, which restricts further growth of the organism [38,56]. In general metal complexes are more active than the ligands because metal complexes may serve as a vehicle for activation of ligands as the principle cytotoxic species.

4.10. Antioxidant activity

The antioxidant activity of the compounds is evaluated by using the scavenging ability of the stable DPPH (2, 2′-diphenyl-1-picryl hydrazyl) radical. The scavenging capacity of $DPPH^{\bullet}$ was determined spectrophotometrically. DPPH (0.004%) was prepared in methanol and 1 ml of 0.004% DPPH methanol solution was mixed with 2 ml of (2 mg/ml) methanol compound mixture. The reaction tubes were wrapped in aluminium foil and kept in dark for 30 min. The reduction of the $DPPH^{\bullet}$ was monitored by observing the decrease in absorbance at 517 nm using UV–Vis spectrophotometer with solvent and DPPH as blank. The percentage inhibition was calculated using the following formula

$$\text{Percent (\%)inhibition of DPPH activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ = absorbance of $DPPH^{\bullet}$ in methanol without an antioxidant, A_{sample} = absorbance of $DPPH^{\bullet}$ in the presence of an antioxidant.

The percentage antioxidant activity of the ligand and its metal complexes are given in Table 5. The antioxidant activity of synthesized compounds was determined in terms of either radical scavenging capacity against stable radical $DPPH^{\bullet}$ or hydrogen donation. The change in color of the DPPH from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm using UV–Vis spectrophotometer. From the experimental results, the enhanced antioxidant activity is observed for all the metal complexes than the free ligand. (Figure. 6) Among all the metal complexes, $[Cu(C_{32}H_{24}N_8O_4)Cl_2]$ and $[Ni(C_{32}H_{24}N_8O_4)Cl_2]$ showed more antioxidant activity. The values imply that the activity of the compounds follows the order $[Cu(C_{32}H_{24}N_8O_4)Cl_2]$ > $[Co(C_{32}H_{24}N_8O_4)Cl_2]$ > $[Ni(C_{32}H_{24}N_8O_4)Cl_2]$ > $C_{32}H_{24}N_8O_4$ (L).

Table 5. Antioxidant activity of Schiff base and its metal complexes

Compound	Antioxidant activity (%)
$C_{32}H_{24}N_8O_4$ (L)	23.7
$[Ni(C_{32}H_{24}N_8O_4)Cl_2]$	28.5
$[Cu(C_{32}H_{24}N_8O_4)Cl_2]$	38.4
$[Co(C_{32}H_{24}N_8O_4)Cl_2]$	33.1

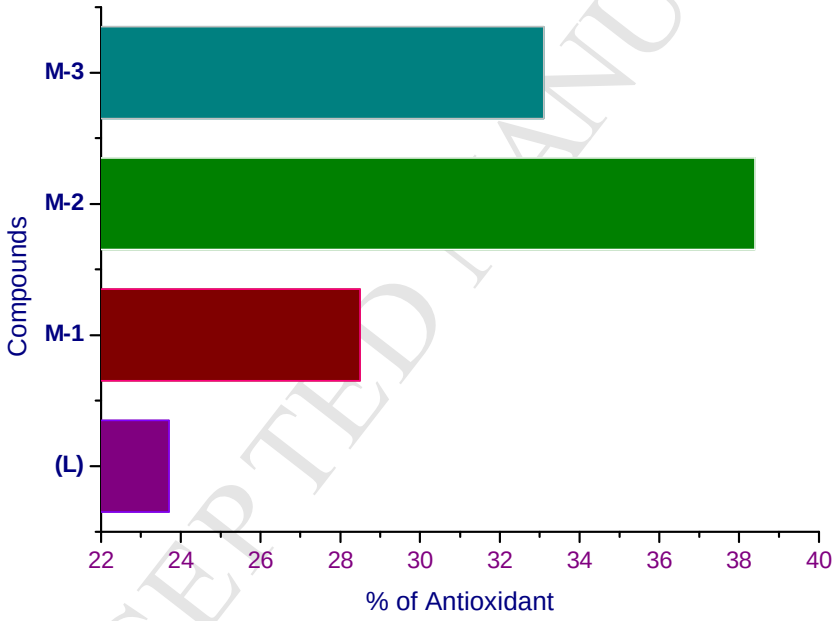


Figure 6. Antioxidant activity of macrocyclic ligand and its metal complexes

5. Experimental protocols

All the reagents were of the best grade available and used without further purification. Metal salts (Merck), O-phthalaldehyde (Merck), diethyl terephthalate and hydrazinhydrate (Aldrich) were used for the synthesis. All necessary precautions were taken to exclude moisture during the synthesis and handling of the compounds. Fourier transform infrared (FTIR) spectra of

the ligand and its metal complexes were recorded in the spectral range 4000-400 cm−1(using KBr) with a Perkin-Elmer Series 2400 apparatus and FT-IR/FIR Perkin Elmer (Frontier) spectrometer in the range 700-30 cm−1(using CsI) respectively. The ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer in DMSO against tetramethylsilane (TMS) as internal reference. Electronic spectra were recorded on a Perkin-Elmer spectrophotometer. The microanalysis of C, H and N were estimated by Perkin-Elmer 2400 CHNSO elemental analyser. X-ray diffraction of the synthesised compounds were carried out in powder form using RIGAKU ULTIMA IV diffractometer system at 25˚C using characteristic Cu monochromatic radiation and the generator settings as 30 mA, 40 kV. Magnetic susceptibility measurements were done using Gouy balance.

6. Conclusion

Macrocyclic hydrazide ligand based Ni(II), Cu(II) and Co(II) metal complexes were synthesized. The physical and analytical data revealed a single metal ion mononuclear six coordinated octahedral geometry of the complexes, with general formula [M($C_{32}H_{24}N_8O_4$) Cl_2]. Antimicrobial and antioxidant activity profile of the tested compounds revealed that the metal complexes show enhanced biological activity compared to the free ligand, which could be attributed to the presence of metal ions in the coordination sphere. Any speculation about the enhanced biological activity and possible mechanism of action of these complexes, however, warrent further studies.

Supporting Information

All additional information pertaining to the synthesised compounds is available in Supplementary Information.

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HIGHLIGHTS

- Three new macrocyclic transition metal complexes of Ni^{2+}, Cu^{2+} and Co^{2+} were synthesized.
- Synthesized compounds were characterized by various physical and spectroscopic techniques.
- IR spectrum of the ligand indicates the coordination of the azomethine nitrogen atoms.
- An octahedral geometry has been assigned for Ni^{2+}, Cu^{2+} and Co^{2+} complexes.
- Antimicrobial activities of the ligand and its complexes, as growth inhibiting agents, have been screened in vitro against different species of bacteria and fungi.
- The complexation led to a remarkable increase in antimicrobial activity.