



Synthesis, Characterization and Antimicrobial Studies of N^1 -[(1*E*)-1-(2-Hydroxyphenyl)ethylidene]-2-oxo-2*H*-chromene-3-carbohydrazide and its Metal Complexes

K. SIDDAPPA*, K. MALLIKARJUN, TUKARAM REDDY,
M. MALLIKARJUN, C.V.REDDY# and MAHESH TAMBE

*Department of Studies and Research in Chemistry,
Gulbarga University, Gulbarga-585106, Karnataka, India.

#C. B. Arts and Science Degree College,
Bhalki, Dist. Bidar, Karnataka, India.

siddappa_65@rediffmail.com

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Abstract: A new complexes of the type ML, M'L and M'L [where M=Cu(II), Co(II), Ni(II) and Mn(II), M'=Fe(III) and M''=Zn(II), Cd(II) and Hg(II) and L= N^1 -[(1*E*)-1-(2-hydroxyphenyl)ethylidene]-2-oxo-2*H*-chromene-3-carbohydrazide (HL)] Schiff base have been synthesized and characterized by elemental analysis, magnetic susceptibility, molar conductance, IR, ^1H NMR, UV-Visible and ESR data. The studies indicate the HL acts as doubly monodentate bridge for metal ions and form mononuclear complexes. The complexes Ni(II), Co(II), Cu(II) Mn(II) and Fe(III) complexes are found to be octahedral, where as Zn(II), Cd(II) and Hg(II) complexes are four coordinated with tetrahedral geometry. The synthesized ligand and its metal complexes were screened for their antimicrobial activity.

Keywords: Coumarin, Metal complexes, Antimicrobial activity, Spectral data.

Introduction

Coumarins are well-known natural products displaying a broad range of biological activities¹. Owing to their diverse bioactivities *viz.* anticoagulant^{2,3} antibacterial, antifungal⁴, antibiotic⁵, spasmolytic⁶, anthelmintic⁷, diuretic⁸, anti-inflammation⁹, antitubercular agents¹⁰, anti-histaminic agents¹¹, antidepressant¹² and antimalarial¹³. Hence it was thought to undertake such study. Chelating ability of coumarin derivatives have been studied to suggest

their use as a chelating agents^{14,15}. The literature survey reveals that the reaction of 2-oxo-2*H*-chromene-3-carbohydrazide and 2-hydroxy acetophenone Schiff base have not been reported so far. On the basis of this, we have synthesized ligand (HL) and its metal complexes and their coordination behavior were investigated.

The present paper deals with the synthesis, spectral, magnetochemical and biological studies of metal complexes with the following ligand (HL) (Figure 1).

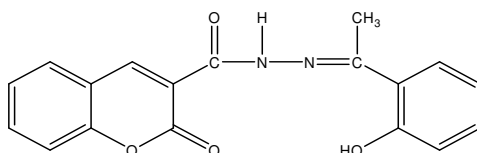


Figure 1. Structure of ligand, HL

Experimental

All the chemicals used were of analytical grade and were used without further purification.

Synthesis of ligand (HL)

The Schiff base ligand was prepared by condensation of 2-oxo-2*H*-chromene-3-carbohydrazide (0.1 M) and 2-hydroxy acetophenone (0.1 M) in ethanol was refluxed on water bath for 5-6 hours in presence of few drops of acetic acid. The reaction mixture was cooled to room temperature. The separated Schiff base was filtered, washed with hot alcohol and recrystallized from alcohol to get a pure sample. The purity of the Schiff base HL (Figure 2) was checked by TLC.

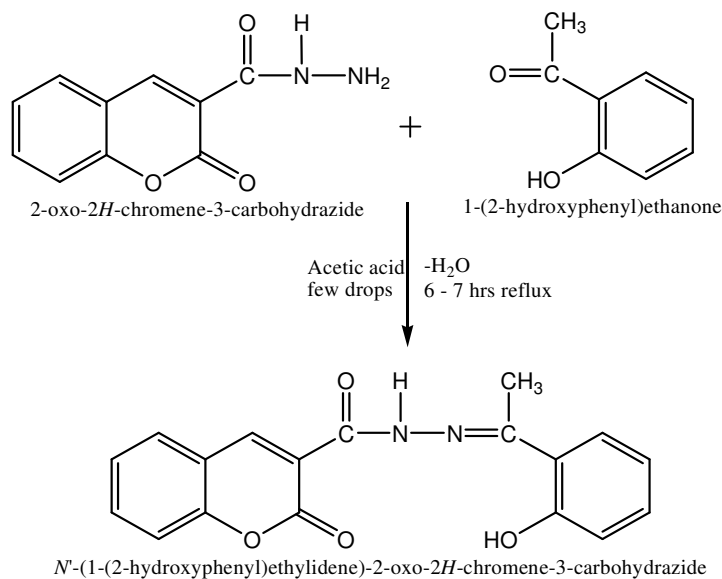


Figure 2. Synthesis of ligand, HL.

Preparation of metal complexes

A solution of 0.01 mole of metal chloride in ethanol was mixed with ethanolic solution of 0.01 mole of HL ligand and refluxed for 3-4 h on water bath to get clear solution. 0.5 g of excess sodium acetate was added to the reaction mixture to adjust the pH 7-8 of the solution. The reaction mixture was further refluxed for 2 h more. The resulting mixture was decomposed by pouring into 100 mL distilled water with constant stirring. The suspended solid complex was allowed to settle and collected by filtration. Then washed with sufficient quantity of distilled water and with little hot ethanol to apparent dryness and dried in a vacuum over anhydrous calcium chloride in a desiccator (Yield, 60-78 %).

Analysis

The complexes were analyzed for their metal and chloride contents by standard methods¹⁶.

Physical measurements

Infrared spectra of the ligand and its metal complexes in KBr pellets were recorded in the spectral range 4000-350 cm^{-1} with Perkin Elmer Spectrum one FT-IR Spectrometer. UV-Visible spectra were recorded on an Elico.SL-164 Double Beam UV-Visible Spectrophotometer in the range of 200-1200 nm. Magnetic susceptibilities were measured on a Guoy Balance at room temperature using HgCo(NCS)_4 as calibrant. The molar conductance of the complexes were measured on ELICO CM-82 conductivity bridge in DMF solution at conc. $\sim 10^{-3}$ M. ^1H NMR spectra were recorded on AMX-400 NMR spectrometer, using TMS as internal standard and DMSO as a solvent. The elemental analysis (CHN), ESR Spectra recorded on STIC Cochin.

Results and Discussion

All the complexes were sparingly soluble in common organic solvents but soluble in DMF, DMSO and acetonitrile. The analytical data indicates that the complexes are agree well with 1:1 metal to ligand stoichiometry for Fe(III), Zn(II), Cd(II) and Hg(II) and 1:2 for Cu(II), Co(II), Ni(II) and Mn(II) complexes shown in Table 1. The observed molar conductance (Table 1) values measured in DMF solution fall in the range (12-20 $\text{Ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$). These observed values of the molar conductance are well within the expected range for non-electrolytic¹⁷.

Magnetic susceptibility

The magnetic susceptibility measurements of the complexes were performed at room temperature (Table 1). The magnetic moment value for Cu(II) complexes of the ligand HL is 1.97 B.M. The copper atom is less than the normal value¹⁸ (1.84-2.20 B.M.). The lowered magnetic moment value observed for Cu(II) complex under present study is due to distorted octahedral geometry^{19,20}. On the other hand Ni(II) and Co(II) complex have shown magnetic moment value 2.97 and 4.88 B.M respectively. This indicates octahedral geometry²¹ for their Ni(II) and Co(II) complexes. The complexes of Mn(II) and Fe(III) exhibited the magnetic moments values 5.65 B.M and 5.79 B.M respectively²². These values indicates that the complexes are high-spin type paramagnetic, it lies within the octahedral range which very close to spin value 5.90 B.M as the ground term is $^6\text{A}_{1g}$ and thus supports the octahedral geometry²³.

Table 1. Analytical, magnetic susceptibility, molar conductance of the ligand [HL] and its metal complexes.

Ligand / Complex	Mol. Wt.	M.P. °C	Yield, %	Found / (Calculated) %					μ_{eff} B.M.	Molar Conductance Λ_m Ohm ⁻¹ cm ² mol ⁻¹
				M	C	H	N	Cl		
C ₁₈ H ₁₄ N ₂ O ₄	322.32	205	80	-	67.07 (67.21)	4.38 (4.44)	8.69 (8.77)	-	-	-
[Cu(C ₃₆ H ₂₆ N ₄ O ₈)]	706.16	280	70	9.00 (8.97)	61.23 (61.46)	3.71 (3.81)	7.93 (7.90)	-	1.97	17
[Co(C ₃₆ H ₂₆ N ₄ O ₈)]	701.55	300	65	8.40 (8.49)	61.63 (61.72)	3.74 (3.81)	7.99 (7.93)	-	4.88	20
[Ni(C ₃₆ H ₂₆ N ₄ O ₈)]	701.31	285	67	8.37 (8.28)	61.65 (61.73)	3.74 (3.86)	7.99 (7.96)	-	2.97	20
[Mn(C ₃₆ H ₂₆ N ₄ O ₈)]	697.55	305	70	7.88 (7.91)	61.99 (61.88)	3.76 (3.82)	8.03 (7.99)	-	5.65	14
[Fe(C ₁₈ H ₁₃ N ₂ O ₄ Cl ₂).H ₂ O]	466.07	290	68	11.98 (11.85)	46.39 (46.28)	3.24 (3.32)	6.01 (6.14)	-	5.79	18
[Zn(C ₁₈ H ₁₃ N ₂ O ₄)Cl]	422.10	300	60	15.49 (15.55)	51.21 (51.39)	3.10 (3.21)	6.64 (6.52)	8.40 (8.44)	Diamag	13
[Cd(C ₁₈ H ₁₃ N ₂ O ₄)Cl]	469.20	280	65	23.93 (23.99)	46.08 (46.29)	2.71 (2.86)	5.97 (5.83)	7.56 (7.61)	Diamag	16
[Hg(C ₁₈ H ₁₃ N ₂ O ₄)Cl]	557.30	310	78	35.99 (35.86)	38.79 (38.85)	2.35 (2.49)	5.03 (5.16)	6.36 (6.38)	Diamag	12

Electronic spectra

The electronic spectral data of Cu(II), Co(II) Ni(II), Mn(II) and Fe(III) complexes were recorded in DMF as shown in Table 2. They have been studied with the view to obtain more information on stereochemistry of the complexes and to procure more support for the conclusion, deduced with the help of magnetic data. The light green colored Cu(II) complex exhibits a broad asymmetric band in the region 16528-12422 cm^{-1} with maxima at 14470 cm^{-1} in an distorted octahedral geometry²⁴. The broadness of the band may be due to dynamic Jahn-Teller distortion and is assigned to ${}^2T_{2g} \rightarrow {}^2E_g$ transitions.

Table 2. Electronic spectral data, ligand field parameters of Cu(II), Co(II) Ni(II), Mn(II) and Fe(III) coumarin complexes.

Complexes	ν_1	ν_2 , cm^{-1}	ν_3	Dq, cm^{-1}	B^1	β	$\beta\%$	ν_2/ν_1	ν_3/ν_2	LFSE k cal, mol^{-1}
[Cu(C ₃₆ H ₂₆ N ₄ O ₈)]		12422-16528	1447	-	-	-	-	-	-	24.80
[Co(C ₃₆ H ₂₆ N ₄ O ₈)]	10152	16260	20618	869	945	0.973	2.67	1.601	1.268	14.89
[Ni(C ₃₆ H ₂₆ N ₄ O ₈)]	11049	15302	26115	933	895	0.860	13.94	1.385	1.706	31.98
[Mn(C ₃₆ H ₂₆ N ₄ O ₈)]	12795	16025	19305	855	861	0.816	18.03	1.252	1.2046	14.67
[Fe(C ₁₈ H ₁₃ N ₂ O ₄ Cl ₂).H ₂ O]	12906	15978	19624	853	885	0.838	16.11	1.238	1.228	14.62

The Co(II) complex (light purple) of the electronic absorption bands appears at 10152 and 20618 cm^{-1} , due to ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(v_1)$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)(v_3)$ transition respectively, in an octahedral environment^{25,26}. The bands due to the ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)(v_2)$ transition could not be observed because of its very low intensity. However the position of the ν_2 band has been computed (16260 cm^{-1}) by the equation ($\nu_2 = \nu_{1+} + 10Dq$). The intense band around 30000 cm^{-1} may be a charge transfer band. The ligand field parameter such as Dq, B^1 , β and $\beta\%$ have been calculated by using band-fitting equation given by Underhill and Billing²⁷, the crystal field splitting energy (Dq) value at 869 cm^{-1} . These values are well within the range reported are most of the octahedral Co(II) complexes. The Co(II) complex under present investigation process interelectronic repulsion parameter (B^1) 945 cm^{-1} . The Racha parameter (B) is less than free ion value (971) suggesting a considerable orbital overlap and delocalization of electrons on the metal ion. The nephelauxetic ratio (β) for the present Co(II) complex is 0.973. This is less than one, suggesting partial covalency in the metal ligand bond. The values Dq, $\beta\%$, LFSE and ν_2 / ν_1 (Table 2) suggest the octahedral geometry for Co(II) complex²⁸.

The electronic spectrum of Ni(II) complex shows two bands at 15302 and 26115 cm^{-1} assignable to ${}^3A_{2g} \rightarrow {}^3A_{1g}(F)(v_2)$ and ${}^3A_{2g} \rightarrow {}^3T_{1g}(P)(v_3)$ transitions respectively, in an octahedral environment²⁹. The lowest band ν_2 (10 Dq) was not observed due to limited range of the instrument used. However, it is calculated by using equation suggested by Billing and Underhill. Racha parameter B^1 is less than the free ion value of 1040 cm^{-1} indicating the covalent character of the complex. The ratio ν_2/ν_1 and $\beta\%$ are further support the octahedral geometry around the Ni(II) ion³⁰. The Electronic spectra of the Mn(II) complex display absorption bands at 12805-19295 cm^{-1} due to the transition ${}^6A_{1g} \rightarrow {}^4A_{1g}$, ${}^6A_{1g} \rightarrow {}^4T_{2g}(G)$ and ${}^6A_{1g} \rightarrow {}^4E_g(G)$ respectively. The ligand field parameters such as Dq, B^1 , β and $\beta\%$ have been calculated (Table 2). These values suggest the high spin octahedral geometry around the metal ion³¹. The Electronic spectra of the Fe(III) complex observed band at (ν_1) 12906, (ν_2) 15978 and (ν_3) 19624 cm^{-1} due to the transitions ${}^6A_{1g} \rightarrow {}^1T_{1g}$, ${}^6A_{1g} \rightarrow {}^1T_{2g}(G)$ and ${}^6A_{1g} \rightarrow {}^1T_{2g}(D)$ respectively. The ligand field parameters such as Dq, B^1 , β and $\beta\%$ have been calculated (Table 2). These values suggest the high-spin octahedral geometry around the metal ion³².

Infrared spectra

The significant IR bands for the ligand *N*¹-[(1*E*)-1-(2-hydroxyphenyl) ethylidene]-2-oxo-2*H*-chromene-3-carbohydrazide (HL) as well as for its metal complexes and their tentative assignments are compiled and represented in Table 3. The broad band observed at 3471 cm⁻¹ in the IR spectra of the ligand (HL) assigned to ν(OH), which were found to have disappeared in all their respective complexes, there by indicating the involvement of phenolic oxygen is bonding with metal ions through deprotonation³³. The band ν(NH) observed at 3184 cm⁻¹ in ligand and complexes ν(NH) observed at 3181-3189 cm⁻¹ respectively. A strong sharp band observed at 1659 cm⁻¹ is assigned to coumarine ring ν(C=O), which was shifted to 9-32 cm⁻¹ in all complexes³⁴, indicates the involvement of coumarine ring carboxyl in complexation with metal ion³⁵, the band at 1603 cm⁻¹ is assigned to the azomethine ν(C=N) group³⁶, lowering of ν(C=N) 8-20 cm⁻¹ in the complexes as compared to its ligand, is due to reduction of double bond character carbon-nitrogen bond of the azomethine group³⁷.

Table 3. Important spectral IR bands of the ligand [HL] and its metal complexes (cm⁻¹).

Ligand / Complex	νOH	νH ₂ O	νNH	νL-C=O	νC=O	νC=N	νC-O-C	Phenolic νC-O	νM-O	νM-N	νM-Cl
C ₁₈ H ₁₄ N ₂ O ₄	3471	-	3184	1659	1603	1501	1299	1239	-	-	-
[Cu(C ₃₆ H ₂₆ N ₄ O ₈)]	-	-	3189	1656	1594	1491	1302	1246	524	480	-
[Co(C ₃₆ H ₂₆ N ₄ O ₈)]	-	-	3187	1630	1593	1491	1301	1247	522	460	-
[Ni(C ₃₆ H ₂₆ N ₄ O ₈)]	-	-	3181	1610	1589	1493	1300	1245	521	458	-
[Mn(C ₃₇ H ₂₉ N ₄ O ₈)]	-	-	3185	1613	1572	1491	1300	1246	522	463	-
[Fe(C ₁₈ H ₁₃ N ₂ O ₄ C ₁₂ ·H ₂ O)]	-	3381	3186	1618	1573	1485	1303	1269	523	464	360
[Zn(C ₁₈ H ₁₃ N ₂ O ₄)Cl]	-	-	3185	1611	1571	1492	1300	1247	521	461	353
[Cd(C ₁₈ H ₁₃ N ₂ O ₄)Cl]	-	-	3184	1612	1572	1493	1300	1247	522	462	358
[Hg(C ₁₈ H ₁₃ N ₂ O ₄)Cl]	-	-	3186	1614	1573	1491	1301	1243	520	461	364

The band observed at 1239 cm⁻¹ of the ligand is attributed to phenolic ν(C-O) in view of previous observations. This band is shifted to higher frequency and is found in the region 1239-1269 cm⁻¹ (16-30 cm⁻¹) for the complexes. Thus, this further confirms the involvement of ν(C-O-C) in the complex formation. The low frequency skeletal vibrations due to ν(M-O) and ν(M-N) stretching provide direct evidence for complexation. In the present investigation, the bands in the 524-520 cm⁻¹ region for ν(M-O) and 480-461 cm⁻¹ region for ν(M-N) vibration respectively³⁸. The bands due to ν(M-Cl) were observed in the 364-353 cm⁻¹ region and are characteristic of chlorine atom in Zn(II), Cd(II) and Hg(II) complexes is further confirmed by quantitative chloride estimation.

¹H NMR spectra

Spectrum of ¹H NMR in DMSO-d₆ solvent was recorded. In ligand *N*¹-[(1*E*)-1-(2-hydroxyphenyl)ethylidene]-2-oxo-2*H*-chromene-3-carbohydrazide (HL) showed sharp peak at δ 13.8 (S, 1H) due to OH at 2-position of phenyl ring of 2-hydroxy acetophenone moiety has resonated, but in the case of Zn(II) complex which has been disappeared indicating the involvement of phenolic oxygen in the coordination via deprotonation³⁹. The peak appears at δ 2.4 (S, 3H, CH₃) due to the azomethine group in ligand but in case of Zn(II) complex the

peak observed at δ 2.3 (S, 3H, $-\text{CH}_3$). The nine aromatic protons due to coumarine and phenyl rings have resonated in region δ 10.7(m, 9H, Ar-H) as a multiplet, in Zn (II) complex the eight aromatic protons have been observed in the region δ 10.6(m, 8H, Ar-H) as multiplet. The Zn(II) complex suggests coordination of the phenolic OH with metal ion⁴⁰.

ESR spectra

ESR spectrum of Cu(II) complexes of ligand N^1 -[(1E)-1-(2-hydroxyphenyl) ethylidene]-2-oxo-2H-chromene-3-carbohydrazide(HL) scanned at room temperature (Table 4) using DPPH as a standard. It showed a broadened feature without hyperfine splitting due to the dipolar interaction from the ESR spectrum of a set of magnetic parameter g_{\parallel} 2.3955 and g_{\perp} 2.1733. The observed ESR spectrum is characteristic of distorted octahedral geometry g value averaged to overall directions and G which is measure of extent of exchange interaction between metal ion have been calculated. In the present case the value of G was found to be 4.218 according to Hathway⁴¹. If G value is greater than 4, the spin exchange interaction is negligible where as G values less than 4 indicate considerable interaction between metal ions in solid complex clearly indicate that Cu(II) ion in the complex is mono-nuclear nature of the complex.

Table 4. ESR data of the Cu(II) complex of the ligand [HL]

Complexes	g_{\perp}	G_{\parallel}	g_{av}	g_{iso}	G
[Cu(C ₃₆ H ₂₆ N ₄ O ₈)]	2.173	2.395	2.249	2.323	4.218

Antimicrobial Activity

The *in vitro* antibacterial screening of the ligand HL and its complexes was undertaken against the bacteria *Escherichia coli* and *Staphylococcus aureus* by cup-plate method⁴² using nutrient agar as medium. In a typical procedure, molten nutrient agar kept at 45 °C was then poured into Petri dishes and allowed to solidify. Then holes of 5 mm diameter were punched carefully using a sterile cork borer and these were completely filled with test solutions (1 mg/mL in DMF). The plates were incubated for 24 h at 37 °C. The diameter of the zones of inhibition for all the test compounds was measured and the results were compared with the standard drug streptomycin of the same concentration as that of the test compound under identical conditions⁴³.

The antifungal activity of the test compounds was evaluated against the *A.niger* and *A.flavous* by cup-plate method cultured on potato-dextrose agar medium adapting similar procedure described above. The plates were incubated at 37 °C for 48 hours. The diameters of the zone of inhibition for all the test compounds were measured and the results were compared with the standard drug streptomycin and chlotrimazole of the same concentration as that of the test compound under identical conditions⁴⁴.

Since all the test compounds and standard drugs were prepared in freshly distilled DMF, its zone of inhibition was found to be very negligible and taken as zero mm. The antibacterial activity results revealed that the ligand (HL) and its complexes shown weak to good activity (Table 5). The ligand and its Cu(II) Co(II), and Hg(II) complexes shows weakly active with the zone of inhibition 12-14 mm against the both organisms when compared to the standard drug streptomycin. The Ni(II), Mn(II) and Fe(III) complexes shows active and moderate activity as compared to its ligand with zone of inhibition 15-17 mm and Zn(II) and Cd(II) complexes have exhibited good activity with the zone of inhibition 18-20 mm when compared to the standard drug streptomycin.

Table 5. Antimicrobial, Antifungal activity results of the ligand [HL] and its metal complexes.

Compound	Antibacterial activity of zone of inhibition, mm		Antifungal activity of zone of inhibition, mm	
	<i>E. coli</i>	<i>S. aureus</i>	<i>A. niger</i>	<i>A. falvours</i>
$C_{18}H_{14}N_2O_4$	10	-	08	09
[Cu($C_{36}H_{26}N_4O_8$)]	13	13	14	13
[Co($C_{36}H_{26}N_4O_8$)]	14	12	12	14
[Ni($C_{36}H_{26}N_4O_8$)]	16	15	16	17
[Mn($C_{36}H_{26}N_4O_8$)]	15	16	19	20
[Fe($C_{18}H_{13}N_2O_4Cl_2$).H ₂ O]	15	17	12	14
[Zn($C_{18}H_{13}N_2O_4$)Cl]	20	20	15	16
[Cd($C_{18}H_{13}N_2O_4$)Cl]	18	20	15	16
[Hg($C_{18}H_{13}N_2O_4$)Cl]	13	11	18	19
Streptomycin	25	24	-	-
Chlotrimazole	-	-	24	26
DMF (Control)	0	0	0	0
Bore size	08	08	08	08

The antifungal activity, results revealed that the ligand and its Cu(II), Co(II), Ni(II), Zn(II), Cd(II), Hg(II), Mn(II) and Fe(III) complexes have exhibited weak to good activity. The ligand (HL) and its Cu(II), Co(II) and Fe(III) complexes shows weak activity with the zone of inhibition 12-14 mm when compared to the standard drug chlotrimazole. The Ni(II), Cd(II) and Zn(II) complexes shows moderate activity as compared to its ligand (HL) with the zone of inhibition 15-17 mm respectively.

The Mn(II) and Hg(II) complexes have exhibited good activity with the zone of inhibition 18-20 mm when compared to the standard drug chlotrimazole.

Conclusion

The elemental analysis, magnetic susceptibility, electronic spectra, IR, ¹H NMR and ESR spectra observations projects the following structures for these complexes where in Cu(II), Co(II) Ni(II) Mn(II) and Fe(III) exhibit coordination number of six, Zn(II), Cd(II) and Hg(II) are four coordinated tetrahedral geometry.

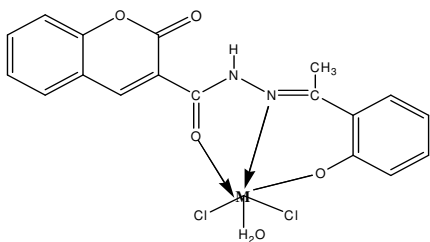


Figure 3. Structure of complexes where M = Cu(II), Co(II), Ni(II) and Mn(II).

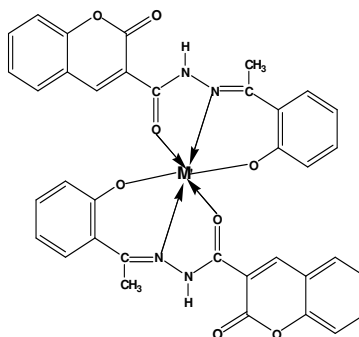
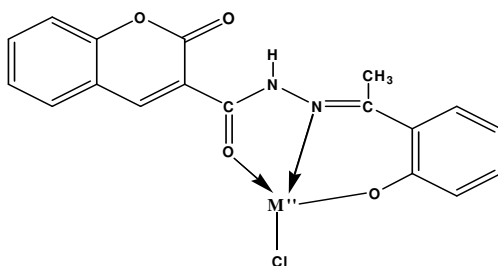


Figure 4. Sturcture of complexes where M' = Fe(III)



where $M'' = \text{Zn(II)}, \text{Cd(II)} \text{ and } \text{Hg(II)}$.

Figure 5. Structure of complexes

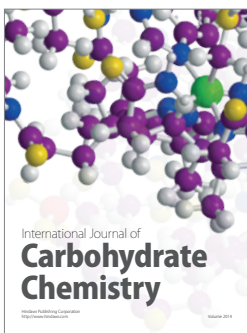
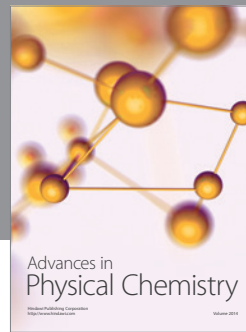
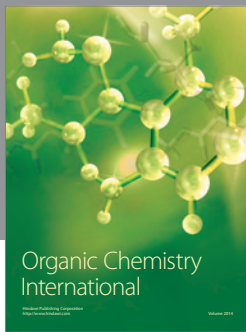
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