

Preparation, Characterization, and Biological Assessment of Heterocyclic Schiff Base Metal Complexes

Abstract

MIIMC, MMIIMC, and CMIIMC are biologically significant Schiff bases formed by the condensation of 3-amino-5-methyl isoxazole with formylchromone. Substituted formylchromones and metal chelates containing Cu(II), Ni(II), Co(II), and Zn(II) are prepared. These were studied using elemental analysis, FTIR, UV-VIS, ^1H , ^{13}C , mass spectra, magnetic susceptibility, conductance tests, and thermogravimetric techniques. The data revealed that the ligands display neutral, bidentate coordination through carbonyl oxygen and azomethine nitrogen. The antibacterial activity of the ligands and metal complexes is determined in vitro against bacteria and fungus using the paper disc method. Metal chelates have significantly higher MIC values against bacteria than ligands. The DNA binding properties of Cu(II) complexes were examined. MTT is used to determine the complexes' anticancer efficacy against HL60 tumour cells. The researchers discovered that the complexes prevented apparent morphological alterations. The inhibition ratio was hastened by increasing the dosage, and it had a strong positive association with the medicine dosage.

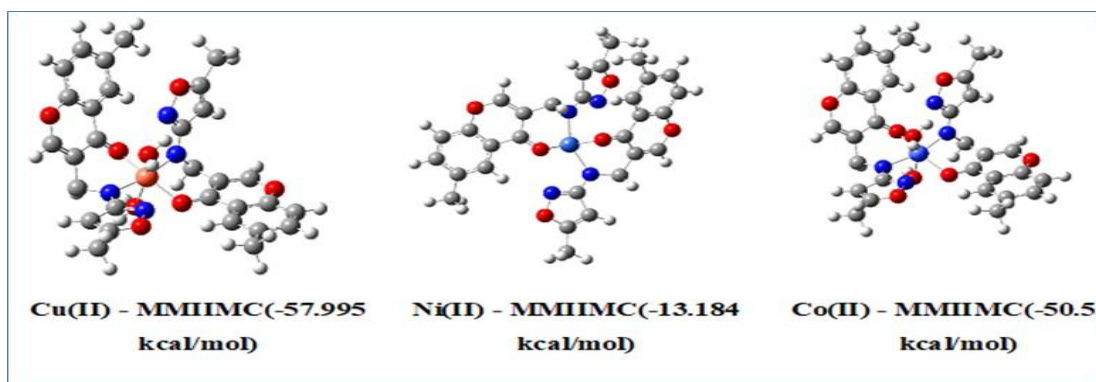
Thermal analysis of complexes showed initial mass loss due to moisture and hydrated water molecules, followed by weight loss at 220°C due to coordinated water molecules, indicating metal oxide breakdown.

Keywords: Biological Assessment, Heterocyclic Schiff Base Metal Complexes, FTIR, UV-VIS, ^1H , ^{13}C , mass spectra.

Introduction

Because of their many biological uses, Schiff bases are highly appreciated in both organic and inorganic chemistry¹. The study of metal complexes involving Schiff bases is gaining popularity among biochemists². "Diverse biological actions are exhibited by isoxazole derivatives, encompassing pharmacological attributes like antibacterial, anti-HIV, and anti-cancer activity, in addition to agrochemical qualities and pesticide and insecticidal applications. Three metal complexes of Schiff bases generated from substituted salicylaldehydes and 3-amino-5-methyl isoxazoles were reported in our study. We found that the activity increased with complexation"^{3,4}. The synthesis, characterization, and biological investigations of Schiff bases and their metal complexes are presented in this study.

- (i) 3-[[5'-methyl-3'-isoxazolyl)imino]methyl]-4H-4-chromenone (MIIMC)
- (ii) 6-methyl-3-[[5'-methyl-3'-isoxazolyl)imino]methyl]-4H-4-chromenone(MMIIMC)
- (iii) 6-chloro-3-[[5'-methyl-3'-isoxazolyl)imino]methyl]-4H-4-chromenone(CMIIMC)



METHODOLOGY:

Physical measurements:

The Varian Gemini Unity Spectrometer was used to record the ^1H NMR spectra of the ligands at 200 MHz and 300 MHz, while the Varian Gemini was used to record the ^{13}C NMR spectra at 100.6 MHz. An equipment called the VG micro mass 7070-H was used to record EI mass spectra. Using KBr pellets in the range of the Perkin-Elmer Infrared model 337, the ligands' and complexes' IR spectra were captured (4000-400 cm^{-1}). Using a Shimadzu UV-VIS 1601 spectrophotometer, the electronic spectra of metal complexes in DMSO were captured. The complexes' magnetic susceptibilities were ascertained by employing the Gouy balance model 7550 and $\text{Hg}[\text{Co}(\text{NCS})_4]$ as a reference material. Using a Mettler Toledo Star system, complexes were subjected to TGA between 0 and 1000°C. The decomposition temperature of complexes and the melting temperatures of the ligands were found. An electronic digital conductivity metre (Digisun model DI-909) was used to measure the conductivity in DMSO solutions (0.001 M). Using microanalytical methods on a Perkin Elmer 240C elemental analyzer, the percentage composition of C, H, and N in the complexes and ligands was ascertained (USA). An EPR Varian-E-112 was used to record the room temperature EPR spectra of copper complexes.

Synthesis of ligands : MIIMC / MMIIMC / CMIIMC

3-Formyl chromone / 3-formyl-6-methylchromone / 3-formyl-6-chlorochromone (0.05 mol) dissolved in methanol was added to a methanolic solution of 3-amino-5-methyl isoxazole (0.05 mol) and refluxed on a water bath for approximately 3 hours. After

cooling, a light yellow crystalline compound separated from the solution, which had been concentrated to half its volume. It was recrystallised from methanol. The purity of the compounds was determined using TLC. Yield: 80-85%.

These Schiff bases were also synthesized by **microwave irradiation and ultrasonic methods**^{5a,5b}. The products obtained were highly pure with good yield compared to the ligands prepared by conventional methods.

Synthesis of metal complexes: (general method) :

The metal and ligands were combined in a 1:2 molar ratio to form the metal complexes. A hot methanolic solution of ligand (0.01 mol) and a hot methanolic solution of matching metal salts (0.005 mol) (MX₂, where M = Cu(II), Ni(II), Co(II), and Zn(II), and X = Chlorides/Acetates) were mixed together with constant stirring. The mixture was refluxed for 2-3 hours on a water bath set to 70-80 degrees Celsius. Colored solid metal complexes formed upon cooling. The products were filtered, washed with cold methanol, and finally dried under vacuum over P₄O₁₀.

Molecular modeling studies: Without X-ray crystal structure data, the three-dimensional structure of the molecules cannot be fully determined. However, recent important breakthroughs in computational chemistry techniques offer an alternate, if approximate, method for estimating the three-dimensional structures of molecules. The probable configurations for the Cu(II), Ni(II), and Co(II) complexes were determined using semiempirical and density functional theory calculations, respectively. PM3, a semiempirical self-consistent field approach, was utilised to calculate the 3D geometries and relative energies of the various isomers of Cu(II), Ni(II), and Co(II) compounds. The most stable structure with the lowest energy among the available structures is deemed to be the most likely.

Molecular modeling structures for Cu(II), Ni(II) and Co(II) complexes :

DNA binding studies:

To ascertain the bonding affinity between DNA and complexes, room temperature absorption spectroscopy titrations were carried out (Cu-MIIMC, Cu-MMIIMC and Cu-CMIIMC). The complex sample (20 μM) and 3 ml of blank solutions comprising buffer were then added to the reference cuvettes (1 cm path length). The initial spectra was captured between 200 and 800 nm. To remove the absorbance of DNA itself during the titration, an aliquot (1–10 μL) of buffered solution (concentration of 5–10 mM in base pairs) was given to each cuvette. The absorption spectra were recorded after the solutions were mixed for five minutes. Until the spectra showed no change, signifying that binding saturation had been reached, the titration procedures were repeated. At the conclusion of

each titration, the variations in metal complex concentration caused by dilution were minimal. At least three repetitions of the absorption spectroscopic titrations were conducted.

RESULTS AND DISCUSSION

A) Characterization of ligands:

Physical properties: Each and every ligand has a yellow colour and is stable in both moisture and air. Their melting points range from 132 to 159°C, and they are soluble in all organic solvents. **Table 1** displays the ligands' analytical data. This is the first report of each of these ligands.

1) MIIMC :

IR (KBr) : 1667 cm⁻¹ν(C=O); 1624 cm⁻¹ ν(C=N).

UV (MeOH) : 352 nm(λ_{max}); 288 nm (λ_{max}).

¹H-NMR (DMSO-d₆) (300 MHz) : δ 7.93 (s, H-2); 6.91-7.65 (m, H-5,6,7,8); 5.94 (CH=N); 5.58(s, H-4'); 2.39 (s, 5'-CH₃).

¹³C -NMR(CDCl₃+DMSO) (100.6MHz) : 177.4(C-4); 169.2(C-3'); 158.9 (C-5') 157.1(C-2); 145.7 (CH=N); 138.4 (C-8a); 135.3 (C-7); 130.8 (C-5); 127.6 (C-4a); 124.2 (C-6); 118.1 (C-8); 106.5 (C-3); 94.4 (C-4'); 12.9(C-5'-CH₃).

MS : m/z at 254 (M⁺) and other fragments are at m/z 188,171,176,121 and 92.

Analysis(%) : Found C, 66.08 ; H, 3.37; N, 11.54. C₁₄H₁₀N₂O₃ requires C, 66.14 ; H, 3.93; N, 11.02.

IR (KBr) : 1665 cm⁻¹ν(C=O); 1628 cm⁻¹ν(C=N).

UV (MeOH) : 354 nm(λ_{max}); 264 nm (λ_{max}).

¹H NMR (DMSO- d₆) (300 MHz): δ 7.71(s, H-2); 7.58(s, H-5); 7.28 (s, H-7); 6.89 (s, H-8); 5.82 (s, CH=N); 5.57 (s, H-4'); 2.42(s, 5'-CH₃); 2.37(s, 6-CH₃).

¹³C NMR(CDCl₃+DMSO) (100.6MHz): 170.8(C-4); 159.2(C-3'); 142.5(C-2); 142.3(C-5'); 135.8(CH=N); 135.7(C-8a); 131.4(C-4a); 126.1(C-5); 122.0(C-6); 117.7(C-7); 106.1(C-3); 101.2(C-8); 93.8(C-4'); 20.4(C-6-CH₃), 12.5 (C-5'-CH₃).

MS : m/z at 268 (M⁺) and other fragments are at m/z 185,159,140,98,77 and 43.

Analysis(%) : Found C, 67.08 ; H, 4.37; N, 10.54. C₁₅H₁₂N₂O₃ requires C, 67.18 ; H, 4.47; N, 10.44.

3) CMIIMC :

IR (KBr) : 1665 cm⁻¹ν(C=O); 1627 cm⁻¹ν(C=N).

UV (MeOH) : 356 nm(λ_{\max}); 260 nm (λ_{\max}).

$^1\text{H NMR(DMSO- }d_6)$ (300 MHz): δ 7.89 (s, H-2); 7.64(s, H-5); 7.41(s, H-7); 6.98 (s, H-8); 5.85 (s, CH=N); 5.59 (s, H-4'); 2.42(s, 5'-CH₃).

$^{13}\text{C NMR(CDCl}_3\text{+DMSO)}$ (100.6MHz): 181.3 (C-4); 171.0 (C-3'); 159.1(C-5'); 154.5 (C-2); 143.3 (CH=N); 134.9 (C-8a); 134.6(C-7); 127.5 (C-4a); 125.9 (C-5); 123.1(C-6); 119.6(C-8); 105.5 (C-3); 93.9 (C-4'); 12.5 (C-5'- CH₃).

MS : m/z 288.5 (M⁺). other fragments are at 263 and 247.

Analysis(%) : Found C, 58.13 ; H, 3.13; N, 9.72. C₁₄H₉N₂O₃Cl requires C, 58.23; H, 3.11; N, 9.72.

Table – 1 Analytical data of the ligands

Compound	M.Wt	M.Formula	M.pt	C%	H%	N%
MIIMC	254	C ₁₄ H ₁₀ N ₂ O ₃	132	66.08	3.37	11.54
MMIIMC	268	C ₁₅ H ₁₂ N ₂ O ₃	159	67.08	4.37	10.54
CMIIMC	288.5	C ₁₄ H ₁₂ N ₂ O ₃ Cl	151	58.13	3.13	9.72

B) Characterization of the Complexes :

Physical characteristics of the complexes :

Every complex is non-hygroscopic and stable at room temperature. They break down at high temperatures when heated. The complexes exhibit solubility in DMSO but insoluble in water.

Elemental analysis :

Table 2 displays the analytical data for the complexes.

The data unequivocally demonstrate that there is good agreement between the theoretical values computed for a 1:2 ratio and the practical values for each complex. As a result, the complexes' allocated composition can be expressed as the table does.

Conductivity:

High conductance values (195-235 Ohm-1.cm².mol⁻¹) are observed in all complexes of MIIMC, MMIIMC, and CMIIMC, suggesting an electrolytic nature in the solvent.

Table – 2 Analytical data metal Complexes

Complex	Formula	M.Wt.	C%	H%	N%	M%
[Cu(MIIMC) ₂ (H ₂ O) ₂] ⁺²	[CuC ₂₈ H ₂₄ N ₄ O ₈]	607.5	55.30 (55.00)	3.95 (3.81)	9.21 (9.89)	10.45 (10.03)
[Cu(MMIIMC) ₂ (H ₂ O) ₂] ⁺²	[CuC ₃₀ H ₂₈ N ₄ O ₈]	635.5	56.64	4.40	8.81	9.99

			(55.94)	(4.00)	(8.81)	(9.09)
$[\text{Cu}(\text{CMIIMC})_2(\text{H}_2\text{O})_2]^{+2}$	$[\text{CuC}_{28}\text{H}_{22}\text{N}_4\text{O}_8\text{Cl}_2]$	676.5	49.66 (49.78)	3.25 (3.51)	8.27 (7.98)	9.38 (9.26)
$[\text{Ni}(\text{MIIMC})_2]^{+2}$	$[\text{NiC}_{28}\text{H}_{20}\text{N}_4\text{O}_6]$	566.7	59.29 (59.00)	3.52 (3.23)	9.88 (9.00)	10.35 (10.59)
$[\text{Ni}(\text{MMIIMC})_2]^{+2}$	$[\text{NiC}_{30}\text{H}_{24}\text{N}_4\text{O}_6]$	594.7	60.53 (60.25)	4.03 (4.03)	9.41 (9.27)	9.87 (9.24)
$[\text{Ni}(\text{CMIIMC})_2]^{+2}$	$[\text{NiC}_{28}\text{H}_{18}\text{N}_4\text{O}_6\text{Cl}_2]$	635.7	52.85 (52.01)	2.83 (2.32)	8.80 (8.79)	9.23 (9.51)
$[\text{Co}(\text{MIIMC})_2(\text{H}_2\text{O})_2]^{+2}$	$[\text{CoC}_{28}\text{H}_{24}\text{N}_4\text{O}_8]$	602.9	55.73 (55.62)	3.98 (3.92)	9.28 (9.32)	9.76 (9.63)
$[\text{Co}(\text{MMIIMC})_2(\text{H}_2\text{O})_2]^{+2}$	$[\text{CoC}_{30}\text{H}_{28}\text{N}_4\text{O}_8]$	630.9	57.06 (57.21)	4.43 (4.49)	8.87 (8.78)	9.33 (9.23)
$[\text{Co}(\text{CMIIMC})_2(\text{H}_2\text{O})_2]^{+2}$	$[\text{CoC}_{28}\text{H}_{22}\text{N}_4\text{O}_8\text{Cl}_2]$	671.9	50.00 (50.02)	3.27 (3.12)	8.33 (8.23)	8.76 (8.27)
$[\text{Zn}(\text{MIIMC})_2]^{+2}$	$[\text{ZnC}_{28}\text{H}_{20}\text{N}_4\text{O}_6]$	573.3	58.60 (58.92)	3.48 (3.51)	9.76 (9.98)	11.39 (11.22)
$[\text{Zn}(\text{MMIIMC})_2]^{+2}$	$[\text{ZnC}_{30}\text{H}_{24}\text{N}_4\text{O}_6]$	601.3	59.87 (59.01)	3.99 (3.61)	9.31 (9.58)	10.85 (10.75)
$[\text{Zn}(\text{CMIIMC})_2]^{+2}$	$[\text{ZnC}_{28}\text{H}_{18}\text{N}_4\text{O}_6\text{Cl}_2]$	642.3	52.31 (52.39)	2.80 (2.87)	8.71 (8.69)	10.16 (10.10)

- Calculated (found)

The determination of chlorides in the metal complexes was carried out by Volhard's method⁶.

Thermal Analysis:

Weight loss was evaluated between ambient and 1000°C, with the heating rates in thermogravimetric analysis (TGA) and differential thermal analysis (DTA) carefully regulated at 10°C min⁻¹ in a nitrogen atmosphere. The TGA curves of the aforementioned complexes show that coordinated water molecules are responsible for the second weight loss at 220°C, while moisture and hydrated water molecules are thought to be lost during the chelate drying process during the first mass loss in the 100–120°C range. Above this temperature, the thermogram is horizontal, with metal oxides being the end products of breakdown. Figure 1 depicts a typical thermogram of Cu-MMIIMC. The presence of water molecules is further confirmed by the endothermic bands evident in the individual DTA curve at temperatures where the TGA curve should lose weight. In addition to endothermic bands, DTA curves of complexes have exothermic bands. These

bands emerged at higher temperatures, indicating phase shift, oxidation, and/or breakdown of the chemical.

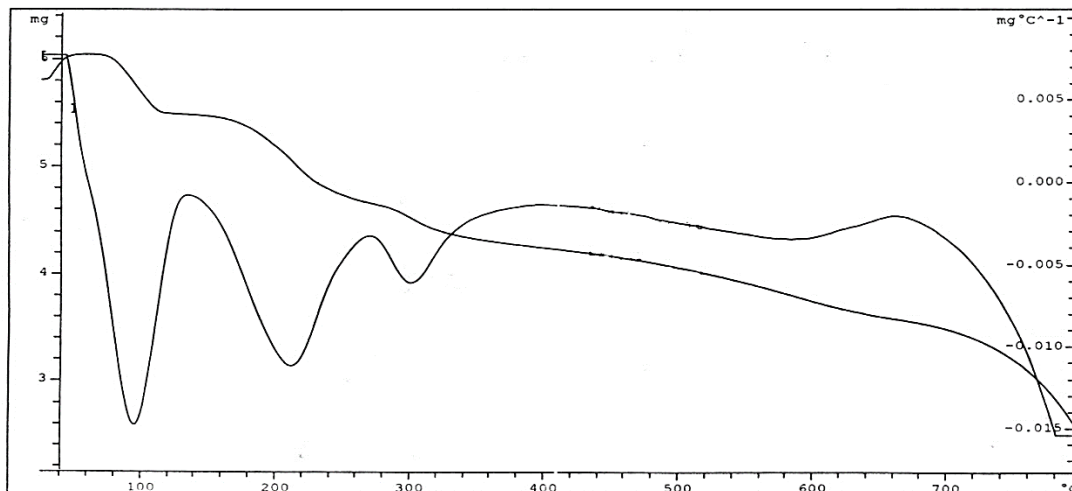


Figure. 1: TGA of Cu(II) MMIIMC

IR spectra: To analyse the Schiff base's binding manner to the metal ion in complexes, free ligand IR spectra are compared to those of their corresponding complexes. Table 3 presents the IR data for all ligands, complexes, and their assignments.

In complexes, the medium intensity band around 1636 cm^{-1} due to $\nu\text{C}=\text{N}$ of all ligands moves to a lower frequency area to the range of 10-25 cm^{-1} , indicating that the nitrogen of azomethine is coordinated with the metal ion 7,8. A band at 1670 cm^{-1} caused by the chromone carbonyl group of MIIMC, MMIIMC, and CMIIMC is moved to a lower frequency area in the complexes, showing that the oxygen in the chromone-carbonyl group contributes in coordination⁹. These findings indicate that the shifts are induced by azomethine nitrogen and carbonyl oxygen coordinating the ligand with the metal atom. Except for Ni(II) and Zn(II), all complexes show a broad diffuse band at 3200-3400 cm^{-1} and another band at 752-758 cm^{-1} in the lower frequency range, which indicates the presence of water. The findings of elemental analysis and T.G.A of complexes confirm this claim. Two new bands formed in the low frequency area about 551-553 cm^{-1} and 440-458 cm^{-1} , caused by $\nu\text{M}-\text{O}$ and $\nu\text{M}-\text{N}$, respectively.

Table – 3IR Absorption frequencies of metal complexes

Complex	$\nu\text{C}=\text{O}$	$\nu\text{C}=\text{N}$	Coordinated Water	$\nu\text{M}-\text{N}$	$\nu\text{M}-\text{O}$
MIIMC	1667	1624	-	-	-

[Cu(MIIMC) ₂ (H ₂ O) ₂] ⁺²	1652	1613	762	440	543
[Ni(MIIMC) ₂] ⁺²	1646	1611	-	430	510
[Co(MIIMC) ₂ (H ₂ O) ₂] ⁺²	1646	1607	764	434	544
Zn(MIIMC) ₂] ⁺²	1650	1577	-	491	524
MMIIMC	1665	1628	-	-	-
[Cu(MMIIMC) ₂ (H ₂ O) ₂] ⁺²	1647	1616	774	420	544
[Ni(MMIIMC) ₂] ⁺²	1659	1617	-	509	430
[Co(MMIIMC) ₂ (H ₂ O) ₂] ⁺²	1637	1613	738	554	461
[Zn(MMIIMC) ₂] ⁺²	1660	1617	-	525	482
CMIIIMC	1665	1627	-	-	-
[Cu(CMIIIMC) ₂ (H ₂ O) ₂] ⁺²	1645	1615	775	467	531
[Ni(CMIIIMC) ₂] ⁺²	1653	1604	-	486	544
[Co(CMIIIMC) ₂ (H ₂ O) ₂] ⁺²	1640	1611	772	472	551
[Zn(CMIIIMC) ₂] ⁺²	1662	1580	-	462	545

Magnetic susceptibility and Electronic spectra:

Table 4 lists the electronic spectra and magnetic moments of the metal complexes.

Cu(II) complexes have magnetic moment values ranging from 1.87 to 1.98 B.M., which are typical of distorted octahedral complexes. The latest results show that all Cu(II) complexes have a single broad band in the region of 18,920 cm⁻¹ due to the transition between 2E_g and 2T_{2g}, suggesting tetragonal geometry. Cu (II) complexes with tetragonal or square planar structures are expected to create three bands. However, in tetragonal complexes, these three bands usually overlap to produce a single broad absorption band¹¹. Table 4 lists the electronic spectra and magnetic moments of the metal complexes.

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The Ni(II) complexes of MIIMC, MMIIMC, and CMIIIMC were discovered to be diamagnetic, with two bands about 17027-19980 cm⁻¹ and another around 27050-27777 cm⁻¹. The band at 17,027 - 19980 cm⁻¹ corresponds to 1A_{1g} → 1A_{2g}, which falls within the range of a transition proposed for square planar Ni(II) complexes (12,13). The other

band that appears around 27050-27777 cm⁻¹ in these compounds may be due to charge transfer transition.

The magnetic moments of the current Co(II) complexes vary from 4.82 to 5.05 B.M., having a high-spin octahedral structure. The Co(II) complexes show three distinct peaks at 12093-14692 cm⁻¹, 19074-23125 cm⁻¹, and 25411-29012 cm⁻¹, which correspond to the transitions 4T1g(F) → 4T2g (F) (ν₁), 4T1g(F) → 4A2g (F) (ν₂), and 4T1g(F) → 4T1g (P) (ν₃) of octahedral geometry¹⁴. The complexes' ν₂ / ν₁ values range from 1.80 to 2.2015, indicating an octahedral shape.

Table 4 shows the ligand field characteristics and ligand field splitting energies of complexes. “The β values for the current complexes are less than one, indicating significant covalent character of the metal-ligand bonds”¹⁶.

“All Zn(II) complexes have been shown to be diamagnetic in nature. Tetrahedral geometry is the optimal configuration for tetra-coordinated Zn(II) complexes. Based on analytical, conductance, and spectral data, the Zn(II) complexes of MIIMC, MMIIMC, and CMIIMC are attributed to tetrahedral geometry”^{17,18}.

Table – 4 Electronic spectral data, magnetic susceptibility and Ligand field parameters

Complex	Frequency	ν ₂ /ν ₁	B	B'	10 Dq	μ _{eff}
[Cu(MIIMC) ₂ (H ₂ O) ₂] ⁺²	18,920 ; -	-	-	-	-	1.87
[Ni(MIIMC) ₂] ⁺²	19,980 ; 27,777	-	-	-	-	-
[Co(MIIMC) ₂ (H ₂ O) ₂] ⁺²	12,890;20,800;27,300	1.61	642	0.66	7910	5.00
[Zn(MMIIMC) ₂] ⁺²	-	-	-	-	-	-
[Cu(MMIIMC) (H ₂ O) ₂] ⁺²	19607 ; -	-	--	-	-	1.83
[Ni(MMIIMC) ₂] ⁺²	17027; 27050	-	-	-	-	-
[Co(MMIIMC) ₂ (H ₂ O) ₂] ⁺²	13181 ; 22831 ; 27467	1.61	642	0.66	7910	5.00
[Zn(MMIIMC) ₂] ⁺²	- -	-	-	-	-	-
[Cu(CMIIMC) ₂ (H ₂ O) ₂] ⁺²	18520 ; -	-	-	-	-	1.86
[Ni(CMIIMC) ₂] ⁺²	18982 ; 27350	-	-	-	-	-
[Co(CMIIMC) ₂ (H ₂ O) ₂] ⁺²	12980 ;21750 ;26540	1.67	623	0.64	8770	4.91
[Zn(CMIIMC) ₂] ⁺²	-	-	-	-	-	-

ESR spectra: solid state. At ambient temperature, the ESR spectra of all contemporary Cu(II) complexes are anisotropic, having two peaks, one of small intensity in the low field region and the other of significant intensity in the high field region.

Antimicrobial activity: “The biological activity of the ligands MIIMC, MMIIMC, and CMIIMC, as well as their binary complexes with Cu(II), Ni(II), Co(II), and Zn(II), was screened for antibacterial activity against bacteria (E.coli and P. aeruginosa) and fungi

(*A. niger* and *R. oryzae*) using the paper disc method. These samples contain 1mg/ml DMSO. The experimental procedures and findings gained are discussed based on Overtone's idea and Chelation theory”^(20, 21).

Different complexes' activity against different species is dictated by either microbe cell impermeability or microbial ribosome variations. Table 5 compares the biological activity of the synthesised compounds to certain known antibiotics (gentamycin), showing that the free Schiff base ligand and some of its complexes had better activity or a comparable impact. (Figure. 2).

Table – 5 Antimicrobial activity of the complexes

Complex	<i>E.Coli</i>	<i>P.aeruginosa</i>	<i>R.oryzae</i>	<i>A.niger</i>
MIIMC	+	+	+	+
Cu(II)	++	++	+	++
Ni(II)	++	++	++	++
Co(II)	++	+	+	++
Zn(II)	++	+	+	++
MMIIMC	+	+	-	+
Cu(II)	+++	++	++	+++
Ni(II)	+++	++	++	++
Co(II)	++	++	+	+++
Zn(II)	++	++	+	+
Gentamycin	++	++	--	--

High active = +++ (inhibition zone > 15 mm) ; Moderately active = ++ (inhibition zone > 10 mm) ; Slightly active = + (inhibition zone > 5 mm) ; Inactive = -- (inhibition zone < 5mm).

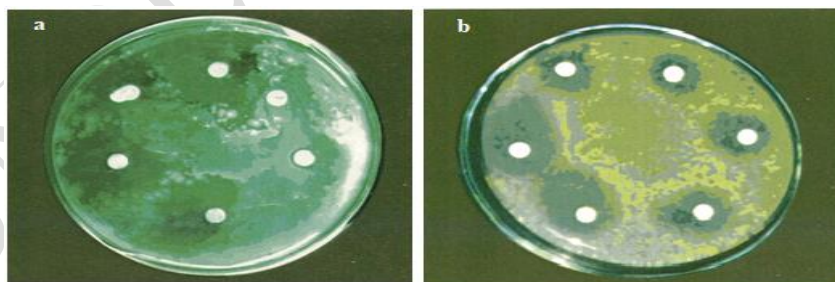


Figure.2 : a) *E. coli* of Cu(II)-MMIIMC, b) *A. niger* of Cu(II)-MMIIMC

DNA binding studies:-

Cu-MIIMC, Cu-MMIIMC, and Cu-CMIIMC absorption spectra are measured with and without CT DNA (at a constant complex concentration of 20 μM). The intrinsic binding constants K_b with CT-DNA were calculated using the following formula in order to

compare the binding strengths of the three complexes: $[DNA]/(\rho b - \epsilon f)$ in contrast to $[DNA]$ ²².

$$[DNA]/(\epsilon_a - \epsilon_f) = [DNA]/(\epsilon_b - \epsilon_f) + 1/(K(\epsilon_b - \epsilon_f))$$

where ρb is the extinction coefficient of the complex fully bound to DNA, ρf is the extinction coefficient of the complex without DNA, and ϵa is the observed extinction coefficient for the CT absorption band at a specific DNA concentration. K_b is the slope-to-intercept ratio in $[DNA]/(\epsilon b - \epsilon f)$ vs. $[DNA]$ graphs. Based on absorbance decay, the intrinsic binding constants (K_b) for 1, 2, and 3 were $2.0 \pm 0.2 \times 10^3$, $3.0 \pm 0.2 \times 10^3$, and $2.2 \pm 0.2 \times 10^3$ M⁻¹, respectively. Complex 2 bonds more firmly than the other two complexes, according to the binding constants. The complexes displayed hypochromism in the subsequent sequence: 2>3>1 .

Anti Canceractivity: To evaluate the Cu-MIIMC, Cu-MMIIMC, and Cu-CMIIMC, HL 60 cells are used. The IC₅₀ values of these compounds are 35.48 ± 1.12 μ g/ml.

1. CELL LINE: In RPMI 1640, human promyelocytic leukaemia (HL60) cells were grown. In a humidified atmosphere of 95% CO₂ at 37 degrees Celsius, the media were supplemented with 10% heat-inactivated FCS, 1 mM NaHCO₃, 2 mM glutamine, and penicillin-streptomycin.

2. TEST CONCENTRATIONS: In order to prepare the stock concentrations, 1 ml of DMSO was used to dissolve 8 mg of each test chemical, and the resulting 200 μ g/ml experimental stock solution was obtained by diluting 25 μ l of the original stock solution to 1 ml. The cultivated cells in the medium were supplemented with different aliquots of experimental stock (final volume of 200 μ l) to attain test concentrations of 10, 20, 40, 60, 80, and 100 μ g/ml..

3. BIOASSAY: HL60 cells were seeded in 96-well microtiter plates at a final density of 2×10^4 cells/well for all studies. Test chemical concentrations ranging from 10 to 100 μ g/ml were applied to the cells. The cytotoxicity was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasolium bromide] test, which was carried out in accordance with Mosmann's (1983)23 protocol. In short, 2×10^4 cells were planted in each well of 96-well plates using 0.1 ml of either RPMI medium or DMEM. Two days following the initial 24-hour period, the vitality of the cells was evaluated by adding 10 μ l of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; 5 mg/ml; stock solution, Sigma) to each well. After that, the plates were incubated for a further four hours at 37°C. Both the media and the formazan blue that developed in the cells were thrown away. Using a spectrophotometer, the rate of colour generation was determined at 570 nm (Spectra MAX Plus; Molecular Devices; supported by SOFTmax PRO-5.0).

Every experiment was conducted using the typical lighting seen in laboratories. The percentage of cell viability inhibition was calculated by comparing the results to the control values (without test compound). In order to get the best straight-line fit, the regression lines were shown after the data was analysed using linear regression. The relevant regression equation was used to get the IC₅₀ (inhibition of cell viability) concentrations.

Cell Morphological Assessment: A phase-contrast microscope was used to look at the morphological anomalies. After a 24-hour treatment, cells showed clear morphological alterations, such as the production of apoptotic bodies and chromatin condensation and fragmentation. The control group, on the other hand, lacked the test substance and had normal, healthy nuclei with no anomalies. While most treated cells in all test doses showed similar signs of apoptosis, the cells exposed to the highest concentration suffered the most damage (**Figure3**).

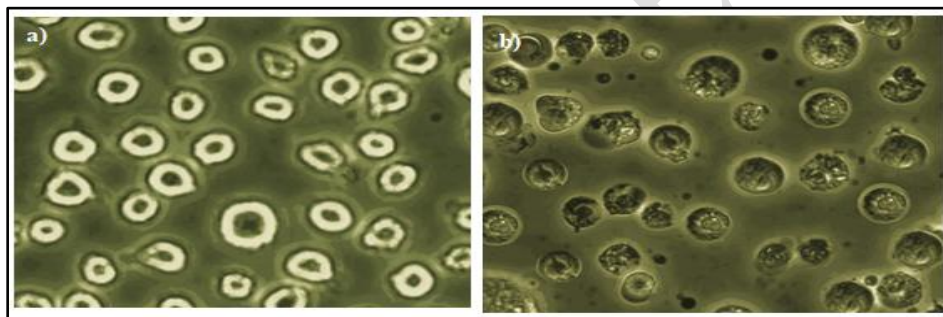


Figure. 3: Morphological changes in HL60 cells treated with and without test compound: a) Control cells with intact nuclei; b) cell membrane blebbing, nuclear fragmentation and chromatin condensation.

Metal chelates from MIIMC, MMIIMC, and CMIIMC have been structurally analyzed, showing a 1:2 metal-ligand stoichiometry. These complexes act as electrolytes in DMSO, with octahedral for Cu (II) and Co(II), square planar for Ni(II), and tetrahedral for Zn (II).

Conclusions:

The structural characteristics of the metal chelates of MIIMC, MMIIMC, and CMIIMC have been determined. In every compound, the metal ligand stoichiometry is 1:2. In DMSO, the ligand complexes mentioned above serve as electrolytes. By connecting between the oxygen of the chromone-carbonyl group and the nitrogen of the azomethine, these ligands function as neutral and bidentate. All of these complexes are attributed to octahedral geometries for Cu(II) and Co(II), square planar geometries for Ni(II), and tetrahedral geometries for Zn(II) complexes based on analytical, conductance, magnetic, and electronic spectrum data. Biological studies of these complexes reveal that these

complexes show better activity compared to their respective ligands. Based on DNA binding studies, the binding constants of the complexes is more due to greater planar area of the complexes. The Cu(II) complexes of these ligands have a therapeutic value against human cancer cell lines especially on leukemia cells.

References:

1. B.S. Tovrog, D.J. Kitko and R.S. Dragom *J. Am. Chem. Soc.*, 98, 5144 (1976).
2. B. Katia, L. Simon, R. Anne, C. Gerard, D. Francoise and M. Bernard, *Inorg. Chem.*, 35, 387 (1996).
3. T.M.D.V. Tomedo Pinho e Melo, 9(10), 2005.
4. Y. Prashanthi, K. Kiranmai, N.J.P. Subhashini and Shivaraj, *Spectrochimica Acta, Part A*, 70/1, 30-35, 2008.
5. a) R.N. Gegye, J.V. Weiv, *Canadian JI. of Chem.*, 76, 525 (1998). b) Li.K.J.T., Han J.S., Yang.J.H., Li.P.S., *Ultrasonic Sonochemistry*, 10, (2003).
6. J.S. Basset, R.C. Denny and J. Mendharm, *Vogel's Text Book of Quantitative Inorganic analysis*, 4th Edn, ELBs, Longman group Ltd, (1978).
7. E. Canpolat, *Polish J. hem.*, 79, 619 (2005).
8. A.A. Solimann and W. Linert, *Thermochimica Acta.*, 333, 67 (1999).
9. K.B. Gudasi and M.S. Patil, *Trans. Met. Chem.*, 31, 580 (2006).
10. J.R. Ferraro, *Low frequency vibrations of inorganic and Coordination compounds*, 1st ed., Plenum Press, New York, 168 (1971).
11. J. Sanmartin, M.R. Bermejo, A.M.G. Deibo, M. Maneiro, C. Lage and A.J.C. Filho, *Polyhedron*, 19, 185 (2000).
12. F.A. Cotton and G. Wilkenson, 'Advanced inorganic chemisry' Wiley-Easetrn Ltd., New Delhi, 3rd ed., 897 (1972).
13. D.U. Warad, C.D. Satish, V.H. Kulkarni and C.S. Bajgur, *Ind.J.Chem.*, 39A, 415 (2000).
14. N. Mondal, D.K. Dey, S. Mitra and K.M. Abdul Malik, *Polyhedron*, 19, 2707 (2000).
15. N.R.S. Kumar, M. Nethiji and K.C. Patil, *Polyhderon*, 10, 365 (1991).
16. T. Sudershan, Ph.D thesis, submitted to Kakathiya University, Warangal, India, 1992.
17. K. Bertoncello, G.D. Fallon, K.S. Murry and E.R.T. Tiekink, *Inorg.Chem.*, 30, 3562 (1991).
18. M. Imran, J. Iqbal, S. Iqbal and N. Ijaz, *Turk J Biol.*, 31, 67 (2007).

19. A.P. Mishra, M. Khare and S.K. Gautam, *Synth.React.Inorg.Met.Org.chem.*, 32, 1689 (2002).
20. Y. Anjaneyulu and R.P. Rao, *Synth.React.Inorg.Met.Org.Chem.*, 16,257 (1986).
21. L. Mishra and V.K. Singh, *Ind.J.Chem.*, 32 (A), 446 (1997).
22. A. Wolfe, G.H. Shimer and T. Meehan, *T. Biochemistry*, 26, 6392 (1987).
23. T. Mosmann, Rapid colorimetric assay for cellular growth and survival : Application to proliferation and cytotoxicity assays. *J. Immunol Methods* 65.

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