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Research Article

Synthesis, Characterization, and Biological Studies of Binuclear Copper(II) Complexes of (2E)-2-(2-Hydroxy-3-Methoxybenzylidene)-^4N-Substituted Hydrazinecarbothioamides

P. Murali Krishna,[1] B. S. Shankara,[2] and N. Shashidhar Reddy[3]

[1] *Department of Chemistry, M. S. Ramaiah Institute of Technology, Bangalore 560 054, India*
[2] *Department of Chemistry, Sri Krishna Institute of Technology, Bangalore 560 090, India*
[3] *Department of Chemistry, S.D.M. College of Engineering and Technology, Dharwad 580 002, India*

Correspondence should be addressed to P. Murali Krishna; muralikp21@gmail.com
and N. Shashidhar Reddy; nshashidharreddy@gmail.com

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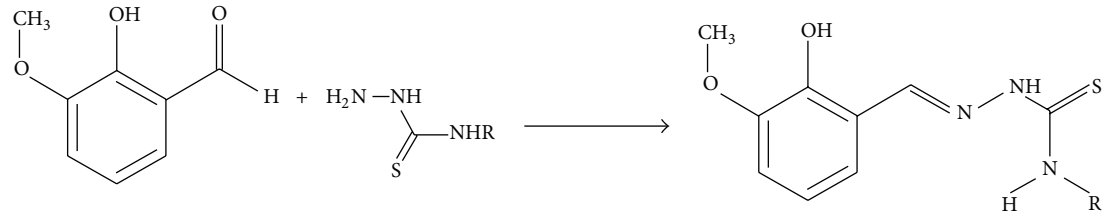
Four novel binuclear copper(II) complexes [**1**–**4**] of (2E)-2-(2-hydroxy-3-methoxybenzylidene)-^4N-substituted hydrazinecarbothioamides, $(OH)(OCH_3)C_6H_4CH{=}NNHC(S)NHR$, where R = H ($\mathbf{L}_1$), Me ($\mathbf{L}_2$), Et ($\mathbf{L}_3$), or Ph ($\mathbf{L}_4$), have been synthesized and characterized. The FT-IR spectral data suggested the attachment of copper(II) ion to ligand moiety through the azomethine nitrogen, thioketonic sulphur, and phenolic-O. The spectroscopic characterization indicates the dissociation of dimeric complex into mononuclear [Cu(L)Cl] units in polar solvents like DMSO, where L is monoanionic thiosemicarbazone. The DNA binding properties of the complexes with calf thymus (CT) DNA were studied by spectroscopic titration. The complexes show binding affinity to CT DNA with binding constant (K_b) values in the order of 10^6 M^{-1}. The ligands and their metal complexes were tested for antibacterial and antifungal activities by agar disc diffusion method. Except for complex **4**, all complexes showed considerable activity almost equal to the activity of ciprofloxacin. These complexes did not show any effect on Gram-negative bacteria, whereas they showed moderate activity for Gram-positive strains.

1. Introduction

Thiosemicarbazones have been emerged as an important class of sulphur and nitrogen containing ligands in the last few decades [1–6] due to their variety of biological activities, such as antitumor [7], antifungal [8, 9], antibacterial [9, 10], and antiviral [11] activities. The biological activity of these compounds depends upon the starting materials and their reaction conditions [12], also related to molecular conformation in particular, which can also be significantly affected by the presence of intra- and intermolecular hydrogen bonding. Thiosemicarbazones usually act as chelating ligands for metal ions, bonding through sulphur (=S) and azomethine (=N–) groups, although in some cases they behave as mono dentate ligands where they bind through sulphur (=S) only [13]. The structural investigations of 2-hydroxy-3-methoxy benzaldehyde thiosemicarbazone ($\mathbf{L}_1$) [14] and its copper(II) [15] and molybdenum(VI) [16–18] complexes were reported, but the structural studies on thiosemicarbazone ligands obtained from substituted thiosemicarbazides and their complexes are worthy to be reported. Therefore, in continuation of ongoing study on thiosemicarbazones and their metal complexes [13, 19–22], we report herein the synthesis, characterization, and biological studies on copper(II) complexes of (2E)-2-(2-hydroxy-3-methoxybenzylidene)-^4N-substituted hydrazinecarbothioamides.



Where R = H, $\mathbf{L}_1$
= CH_3, $\mathbf{L}_2$
= C_2H_5, $\mathbf{L}_3$
= C_6H_5, $\mathbf{L}_4$

Scheme 1

2. Experimental

Thiosemicarbazide, 4-methyl-3-thiosemicarbazide, 4-ethyl-3-thiosemicarbazide, 4-phenyl-3-thiosemicarbazide, and 2-hydroxy-3-methoxy benzaldehyde were of reagent grade purchased from Sigma-Aldrich. All other chemicals were of AR grade and used as supplied. The solvents were distilled before use. Calf thymus DNA was purchased from Genie Bio labs, Bangalore, India.

2.1. Preparation of the Ligands. The ligands were prepared by the following general procedure described in the literature [23]. To a hot ethanol solution (25 mL) of 2-hydroxy-3-methoxy benzaldehyde (10 g, 0.1 mol) in a 250 mL round bottom flask, 5% acetic acid-water solution of thiosemicarbazide (0.1 mol) was mixed and the reaction mixture was refluxed on a steam bath for 30–45 min. The crystalline product which formed was collected by filtration, washed several times with hot water, then ether, and finally dried *in vacuo*. All the ligands were recrystallized from ethanol (Scheme 1).

2.2. Preparation of the Complexes. The metal complexes were prepared by mixing appropriate ligand (2 mol) in DMF and a solution of dihydrated copper(II) chloride (1 mol) in ethanol, that is, in 2 : 1 mole (L : M) ratio. The reaction mixture was refluxed for about 1 hr, during which time a solid complex formed was separated by filtration and washed with hot water, hot ethanol, and finally dried in vacuum desiccators over anhydrous $CaCl_2$.

2.3. Physical Measurement. Elemental analyses were carried out by using a vario EL III elemental analyser. Magnetic susceptibility measurements were recorded on Sherwood scientific magnetic susceptibility balance. High purity hydrated copper sulphate was used as a standard. Molar Conductance measurements were made on an Elico CM-82 conductivity bridge in DMF (10^{-3} M) using a dip-type conductivity cell fitted with a platinum electrode having cell constant 1.0267 scm^{-1}. The ESR spectra of complexes were recorded on a Varian E-122 X-band spectrophotometer at liquid nitrogen temperature in DMSO. The FT-IR spectra of ligands and their complexes were recorded in KBr discs in the range 4000–350 cm^{-1} on Shimadzu FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded in DMSO-d^6 on a Bruker 300 MHz spectrophotometer using TMS as internal standard. The electronic spectra were recorded on an Elico-SL-159 single beam UV-visible spectrophotometer in the range 200–1100 nm in N,N-dimethyl formamide (DMF) (10^{-3} M) solution. FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrophotometer, 6 KV, and 10 mA, using argon as the FAB gas and m-nitro benzyl alcohol as the matrix.

2.4. DNA Binding Experiments. A solution of CT-DNA in 10.5 mM NaCl/5 mM Tris-Hcl (pH 7.0) gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280} of 1.8-1.9, indicating that DNA was sufficiently free of proteins. A concentrated stock solution of DNA was prepared in 5 mM Tris-Hcl/50 mM NaCl in water, pH 7.0, and the concentrations of CT-DNA were determined per nucleotide by taking absorption coefficient (6600 $dm^3\,mol^{-1}\,cm^{-1}$) at 260 nm. Stock solutions were stored at 4°C and were used after no more than four days. Doubly distilled water was used to prepare buffer solutions. Solutions were prepared by mixing the metal copper complex and CT-DNA in DMF medium. After equilibrium (ca. 5 min) the spectra were recorded against an analogous blank solution containing the same concentration of DNA.

The data were then fitted to (1) to obtain the intrinsic binding constant (K_b) [24]. Consider

$$[\mathrm{DNA}] / (\varepsilon_A - \varepsilon_B) = [\mathrm{DNA}] / (\varepsilon_A - \varepsilon_F) + 1/K_b (\varepsilon_B - \varepsilon_F), \quad (1)$$

where ε_A, ε_B, and ε_F correspond to apparent, bound, and free metal complexes extinction coefficients, respectively. A plot of $[\mathrm{DNA}]/(\varepsilon_A - \varepsilon_F)$ versus [DNA] gave a slope of $1/(\varepsilon_B - \varepsilon_F)$ and a Y-intercept equal to $1/K_b(\varepsilon_B - \varepsilon_F)$; K_b is the ratio to the Y-intercept.

2.5. Antimicrobial Activity. The four ligands and their copper complexes were tested *in vitro* against representative Gram-positive bacteria species *Bacillus subtilis*, *Staphylococcus aureus*, and mould two fungal species *Aspergillus niger* and

Table 1: Analytical data, electronic spectra of ligands, and their metal complexes.

Ligand/ complex	Mol. Wt	Yield (%)	Colour	M.P °C	Elemental analysis % found (cal)				μ_{eff}	A_M	Electronic spectral data	
					C	H	N	S	(BM)	(ohm^{-1} cm^2 mol^{-1})	Electronic transition	Assignment
L_1	226	89.30	White	220	47.87 (47.9)	4.95 (4.92)	18.64 (18.60)	14.29 (14.23)	—	—	—	—
1	650	80.40	Green	194	18.52 (18.47)	1.88 (1.89)	7.16 (7.18)	5.47 (5.48)	0.6302	22	16010 24027 32786	d-d transition Charge transfer $\pi \rightarrow \pi^*$ band
L_2	240	86.00	White	232	50.17 (50.19)	5.437 (5.47)	17.51 (17.55)	13.42 (13.40)	—	—	—	—
2	678	84.00	Light green	180	19.57 (19.59)	2.15 (2.13)	6.86 (6.88)	5.27 (5.23)	0.6902	20	16048 24367 32894	d-d transition Charge transfer $\pi \rightarrow \pi^*$ band
L_3	254	71.20	White	226	52.16 (52.15)	5.976 (5.96)	16.54 (16.58)	12.68 (12.65)	—	—	—	—
3	706	70.30	Light green	170	20.65 (20.61)	2.39 (2.36)	6.58 (6.55)	5.13 (5.00)	0.7026	17	17120 23640 32573	d-d transition Charge transfer $\pi \rightarrow \pi^*$ band
L_4	302	74.80	White	206	59.71 (59.78)	5.019 (5.02)	13.95 (13.94)	10.66 (10.64)	—	—	—	—
4	802	72.58	Light green	168	24.48 (24.44)	2.15 (2.05)	5.63 (5.69)	4.38 (4.35)	0.8315	21	16048 24367 32894	d-d transition Charge transfer $\pi \rightarrow \pi^*$ band

Table 2: ^{1}H NMR spectral data of the ligands (chemical shifts in δ (ppm)).

Functional group	$\mathbf{L}_1$	$\mathbf{L}_2$	$\mathbf{L}_3$	$\mathbf{L}_4$
HC=N	9.20 (s, 1H)	9.20 (s, 1H)	9.10 (s, 1H)	9.25 (s, 1H)
CH_3	—	3.10 (s, 3H)	3.55 (s, 3H)	—
$-OCH_3$	3.80 (s, 3H)	3.80 (s, 3H)	3.85 (s, 3H)	3.85 (s, 3H)
–NH	8.40 (s, 1H)	8.40 (s, 2H)	8.45 (s, 2H)	8.25 (s, 2H)
–OH	11.40 (s, 1H)	11.40 (s, 1H)	11.25 (s, 1H)	11.65 (s, 1H)
Aromatics	7.45–8.15 (m, 3H)	6.75–7.65 (m, 3H)	6.65–7.60 (m, 3H)	6.70–7.65 (m, 3H)

Table 3: Mass fragmentation pattern of the ligands.

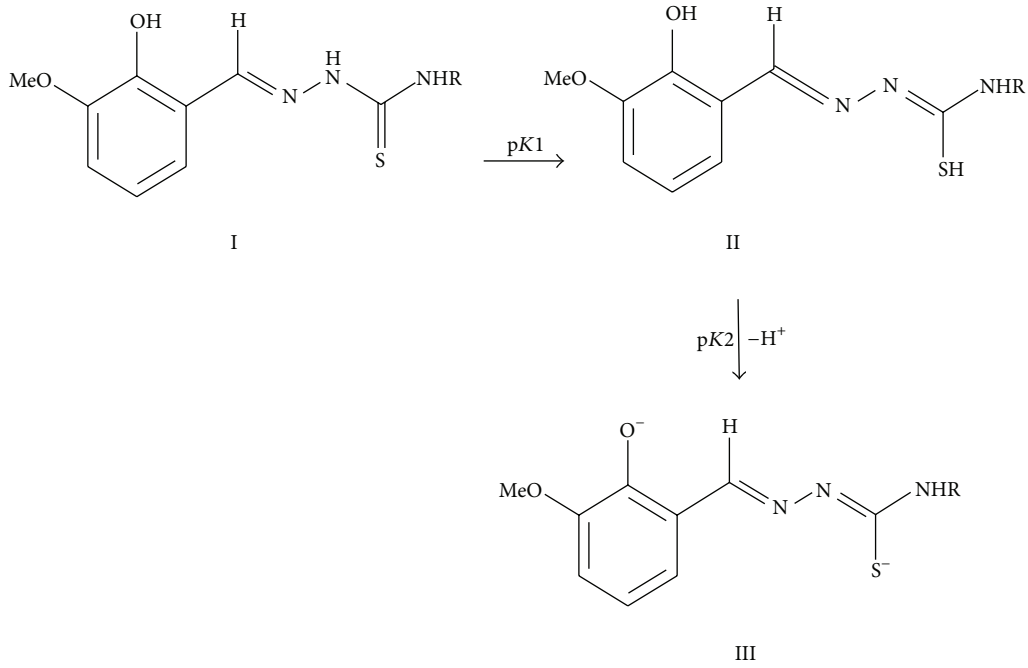
Ligands	m/z	Weight loss	Assignments
$\mathbf{L}_1$	226	—	$C_9H_{11}N_3SO_2$ Molecular ion peak (M^+)
	167	59 (C_2H_3S)	$[C_7H_8N_3O_2]^+$
	151	16 (NH_2)	$[C_7H_6N_2O_2]^+$
	106	45 (CO_2)	$[C_6H_6N_2]^+$
$\mathbf{L}_2$	240	—	$C_{10}H_{13}N_3SO_2$ Molecular ion peak (M^+)
	167	73 (C_3H_5S)	$[C_7H_8N_3O_2]^+$
	151	16 (NH_2)	$[C_7H_6N_2O_2]^+$
	106	45 (CO_2)	$[C_6H_6N_2]^+$
$\mathbf{L}_3$	254	—	$C_{11}H_{15}N_3SO_2$ Molecular ion peak (M^+)
	167	87 (C_4H_7S)	$[C_7H_8N_3O_2]^+$
	151	16 (NH_2)	$[C_7H_6N_2O_2]^+$
	106	45 (CO_2)	$[C_6H_6N_2]^+$
$\mathbf{L}_4$	302	—	$C_{15}H_{15}N_3SO_2$ Molecular ion peak (M^+)
	301	1 (H)	$[C_{15}H_{14}N_3SO_2]^+$

Alternaria alternate by agar well diffusion method [25, 26]. All the bacterial strains were incubated at 37°C for 48 hrs by inoculation into nutrient broth (Difco), and the fungal strains were incubated for 72 hrs by inoculation in to potato dextrose broth (Himedia). The molten media were inoculated with 100 μL of the inoculums and poured into the Petri plate. After medium was solidified, a well was made in the plates with the help of cup-borer (0.85 cm). Then the test compounds were introduced into the well and Petri plates were incubated. Compounds were dissolved in DMSO to get stock solution. Commercially available bactericide Ciprofloxacin and anti-fungal Nystatin were used as standard (100 μg per 100 μL of sterilized distilled water) concomitantly with the test samples. The diameter of inhibition zones (in mm) was determined and data was statistically evaluated by Turkey's pair-wise comparison test. The concentration of DMSO in the medium did not affect the growth of any of the microorganisms tested. All experiments were made in duplicate, and the results were confirmed in three independent experiments.

3. Results and Discussion

3.1. Characterization of the Free Thiosemicarbazones. The thiosemicarbazones (Figure 1) are colourless air stable solids. Their analytical data are given in Table 1. Their pKa values, calculated using the Phillips-Merritt method [27], are 6.0, 6.1, 6.2, and 6.1 for $\mathbf{L}_1$–$\mathbf{L}_4$, respectively. The pKa data indicate that the thiosemicarbazones exist in the thione form in solid state but are converted into the thiol (II) form at lower pH and may lose one proton to bind metal ion in the anionic (III) form at higher pH (Scheme 2). The IR spectra of the ligands showed two medium bands at 3306–3296 cm^{-1} due to terminal ν(NH_2/NHR) vibrational modes. The ligands exhibited a broad medium intensity band in the 2923–2670 cm^{-1} range which is assigned to intermolecular H-bonding vibrations ν(O–H$\cdots$N) which is common in aromatic azomethine compounds containing o–OH groups [24]. In the free ligands, the bands in 1283–1261 cm^{-1} and 3402–3465 cm^{-1} range are attributed to the phenolic ν(C–OH) and ν(OH) group vibrations, respectively [28, 29]. Strong bands observed at 820–831 cm^{-1} and 1540–1527 cm^{-1} are assigned to ν(C=S) and ν(C=N) stretching vibrations, respectively. No band was observed near 2,575 cm^{-1} suggesting the thione form in solid state of the ligands. The ^{1}H-NMR spectra of the ligands were recorded in DMSO-d^6. The chemical shift values in the region δ(11.4–11.8) (s, 1H) are assigned to hydroxyl proton (OH) of the ligands due to the presence of intramolecular hydrogen bonding. The single proton resonances in the ^{1}H-NMR spectra of these ligands that occur at δ(11.10–11.25) have been assigned to azomethine (N=CH–) groups proton. The signals for aromatic ring protons are observed between δ(6.65–8.15) as multiples for the ligands. Methoxy protons appeared at δ(3.80–3.85). The –NH-protons peak appeared at δ(8.25–8.45). ^{1}H NMR spectrum of the ligand $\mathbf{L}_2$ is shown in Figure 2. The ^{1}H-NMR spectral data of the ligands in DMSO-d^6 are given in Table 2. The mass spectra of ligands were performed to determine their molecular weight and fragmentation pattern (Table 3). The mass spectra of $\mathbf{L}_1$–$\mathbf{L}_4$ showed molecular ion peaks M^+ at (m/z) 226, 240, 254, and 302 corresponding to their molecular weights. The ligands $\mathbf{L}_1$–$\mathbf{L}_3$ gave a fragmentation peak at m/z 167 (M^+-59, 73, and 87) from the expulsion C_2H_3S, C_3H_5S, and C_4H_7S species, respectively. This fragment ion undergoes further fragmentation with loss of NH_2 and gave a fragment ion at m/z 151 (M^+-16). Further fragmentation with loss of CO_2 gave a peak at m/z 106 (M^+-45) due to $[C_6H_6N_2]^+$. But the ligand $\mathbf{L}_4$ gave a fragmentation peak at m/z 301 (M^+-1) from the expulsion H species.

3.2. Characterization of the Complexes. The light green coloured copper(II) complexes are stable at room temperature,



Scheme 2

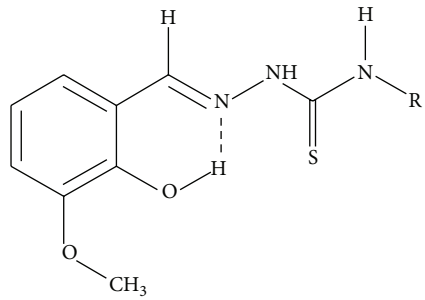


Figure 1: Structure of the thiosemicarbazone ligand.

nonhygroscopic, insoluble in water and common organic solvents, but readily soluble in DMF and DMSO. The physicochemical data of the complexes are summarized in Table 1. The analytical data of complexes support the proposed molecular formula $[Cu_2(L)_2Cl_2]$ (where L is monoanionic thiosemicarbazone). The molar conductivity data indicate that the complexes are nonelectrolytes. As is known, magnetic susceptibility measurements provide information to characterize the structure of the complexes. The magnetic moments of the complexes were measured at room temperature and values indicate that the two copper atoms are held together. Clearly the single unpaired electrons on the copper atoms interact, or couple, antiferromagnetically to produce a low-lying singlet (diamagnetic). The electronic spectral data of Cu(II) complexes recorded in DMF (10^{-3}) solutions are presented in Table 1. Copper(II) complexes have bands between 32573 and 32894 cm^{-1}, assigned to $\pi \rightarrow \pi^*$ transitions of phenyl rings. The charge transfer bands are observed in the range 23600–24400 cm^{-1}, and their broadness can be explained as being due to the combination of $S \rightarrow Cu$ and $N \rightarrow Cu$ LMCT transitions [30]. The Cu(II) complexes exhibited single broad asymmetric d–d bands in the region of 16000–7200 cm^{-1} [31, 32], and the broadness of the band allowed three spin transitions, $^2B_{1g} \rightarrow {}^2A_{1g}$ (ν_1), $^2B_1 \rightarrow {}^2B_{2g}$ (ν_2), and $^2B_1 \leftarrow {}^2E_g$, most probably indicating a square planar configuration. A typical electronic spectrum of complex **1** in DMSO is shown in the Figure 3. The FT-IR spectra of the ligands and their metal complexes are given in Table 4. The FT-IR spectra of the ligands showed two medium bands at 3306–3296 cm^{-1} due to terminal $\nu(NH_2/NHR)$ vibrational modes, and these bands are very similar in the spectra of the complexes suggesting nonparticipation of the terminal $-NH_2$ group in coordination. The ligands exhibited broad medium intensity bands in the 2923–2670 cm^{-1} range which are assigned to the inter molecular H-bonding vibrations $\nu(O–H\cdots N)$. In the complexes, these bands disappear completely on complexation indicating the involvement of O–H group in complex formation. In the free ligands, the bands in 1283–1261 cm^{-1} range are attributed to the phenolic ν(C–OH) group vibration [29], and in the metal complexes these bands are shifted to different frequencies (higher and lower) indicating coordination of oxygen to the metal atoms. The band exhibited at 3402–3465 cm^{-1} can be attributed to the phenolic δ(OH) group vibration [28]. Strong bands observed at 820–831 cm^{-1} and 1540–1527 cm^{-1} are assigned to ν(C=S) and ν(C=N) stretching vibrations, respectively, but on complexation, the stretching frequency of these vibrations are shifted to lower or higher region, which indicates the attachment of attachment of Cu(II) ion to ligand moiety through the thioketonic sulphur and azomethine nitrogen [33]. The nonligand bands at 409–445 cm^{-1} and

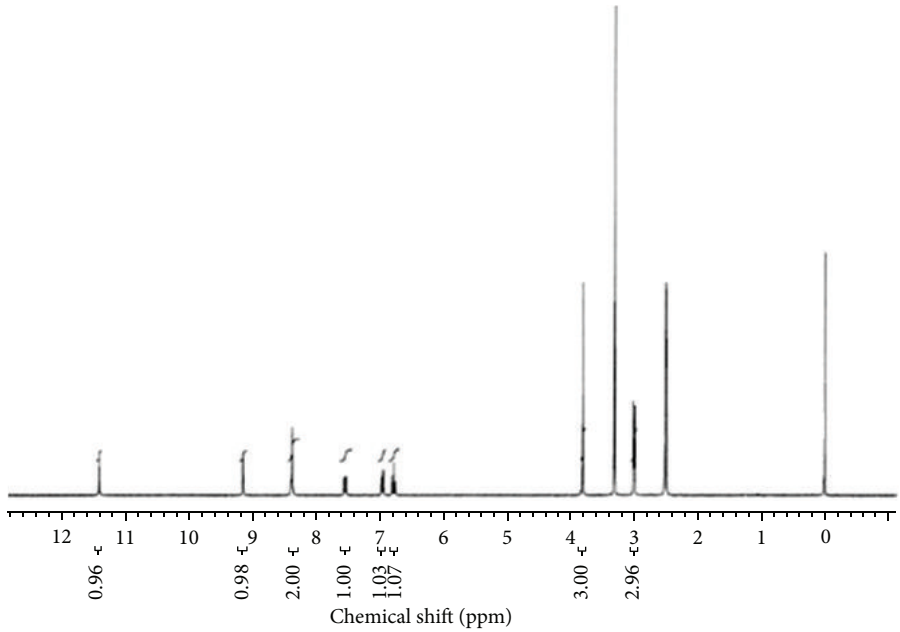


Figure 2: ^{1}H NMR spectrum of the ligand $\mathbf{L}_2$.

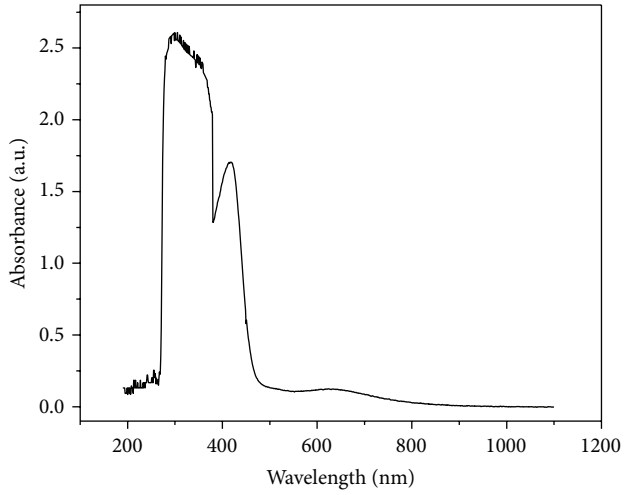


Figure 3: A typical electronic spectrum of complex **1** in DMSO.

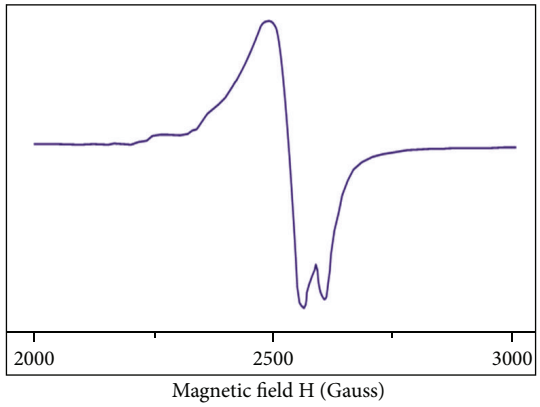


Figure 4: X-Band ESR spectrum of complex **1** at liquid nitrogen temperature (LNT) in DMSO.

374–384 cm^{-1} are tentatively assigned [34] to ν(M–N) and ν(M–S), respectively. For polymeric complexes in which both terminal and bridging metal-halogen linkages present, the ν (M–Cl) stretch for terminal halide is observed at higher wave number side than that for bridging halide [35, 36]. In the present study, the broad and weak intensity nonligand bands assigned to the ν (M–Cl) stretch for the bridging in the region of 342–372 cm^{-1}.

3.3. ESR Spectra. The ESR spectra of the complexes in DMSO at liquid nitrogen temperature exhibit a set of four well resolved signals at low field and one or two signals at high field, corresponding to $g_\parallel$ and $g_\perp$ values, respectively. The $g_\parallel$ and $g_\perp$ values were computed from the spectra using the tetracyanoethylene (TCNE) free radical as the "g" marker. A typical ESR spectrum of **1** is given in Figure 4. The trend in $g_\parallel > g_\perp > 2.0023$ suggests that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital, a characteristic of square planar or octahedral geometry of copper(II) complexes [37]. Neiman and Kivelson [38] have reported that $g_\parallel$ is less than 2.3 for covalent character and greater than 2.3 for ionic character of the metal ligand bond in complexes. As seen in Table 5, the $g_\parallel$ value of all complexes is greater than 2.3, suggesting a small amount of ionic character of the metal ligand bond. The g_{av} value for these complexes is greater than 2, indicating the presence of covalent character [39]. The geometric parameter G, which is a measure of the exchange interaction between copper centers, is calculated using equation $G = (g_\parallel - 2)/(g_\perp - 2)$. If the value of G is

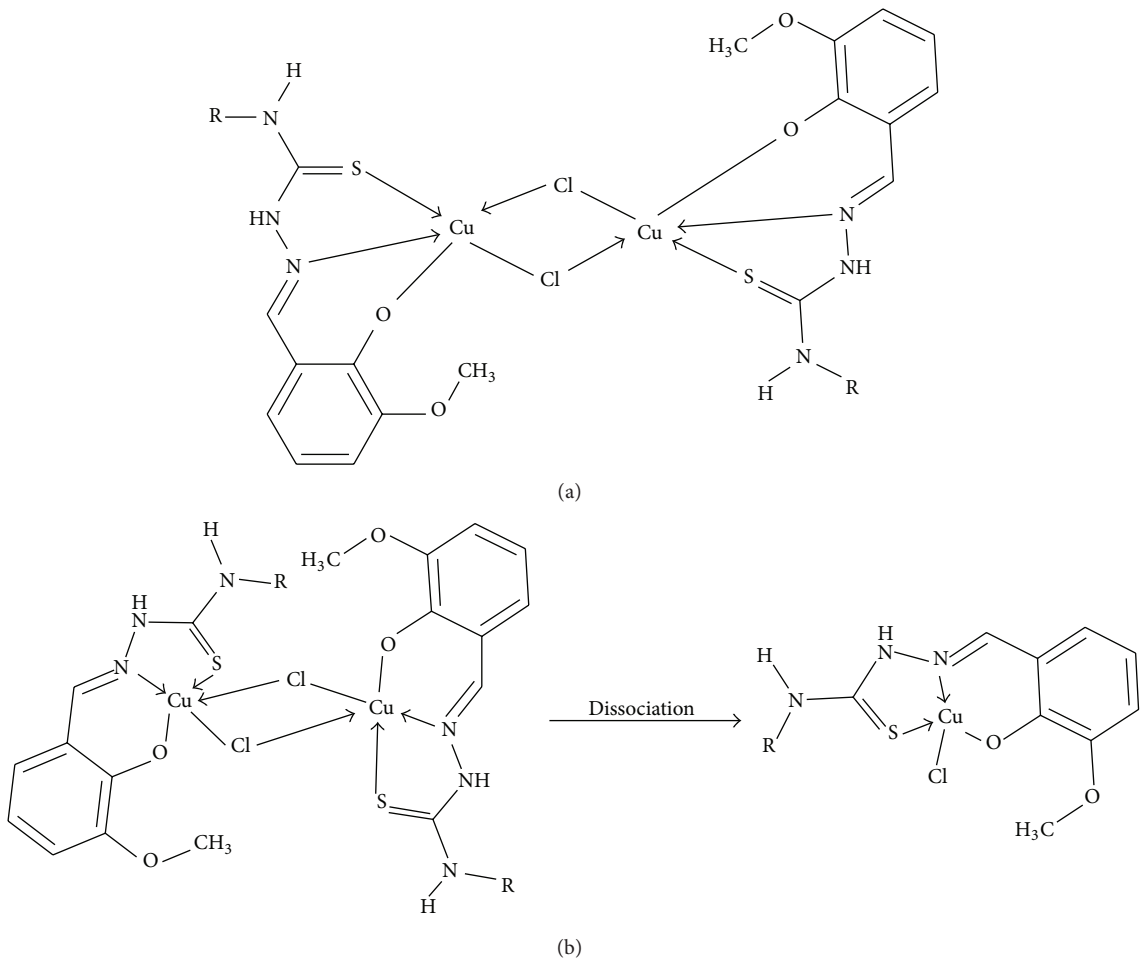


Figure 5: (a) Tentative structure of copper(II) complexes in solid state. (b) Dissociation of dinuclear complex to mononuclear in solution state.

Table 4: Important IR spectral data (cm^{-1}) of the ligands and their metal complexes.

Compound	ν(O–H)	ν(N–H)/NHR	ν(C=N)	ν(C=S)	ν(M–S)	ν(M–N)	ν(M–Cl)
$\mathbf{L_1}$	3460	3293	1579	820	—	—	—
1	3465	3293	1539	635	374	409	356
$\mathbf{L_2}$	3448	3305	1527	831	—	—	—
2	3440	3307	1577	651	379	422	342
$\mathbf{L_3}$	3410	3308	1540	815	—	—	—
3	3402	3309	1552	665	382	431	365
$\mathbf{L_4}$	3452	3296	1539	815	—	—	—
4	3450	3298	1548	673	384	446	372

Table 5: ESR spectral data of Cu(II) complexes.

Complexes	$g_\parallel$	$g_\perp$	g_{av}	G	$A_\parallel \times 10^{-4}$	$f(=g_\parallel/A_\parallel)$	$A_\perp \times 10^{-4}$	$A_{av} \times 10^{-4}$
1	2.3316	2.1013	2.1781	3.3263	158.81	146.82	93.028	114.7
2	2.3232	2.0600	2.1477	2.2070	158.01	147.03	—	—
3	2.3110	2.0251	2.1204	13.5394	167.40	138.05	83.725	111.6
4	2.3110	2.0600	2.1436	2.1847	167.40	138.05	—	—

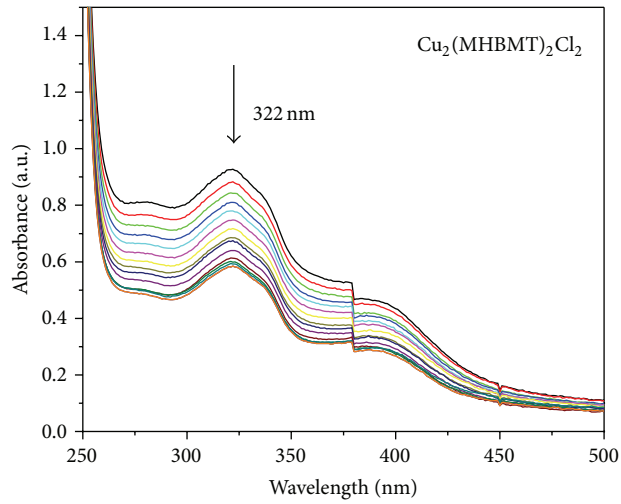


Figure 6: Electronic spectra of complex **2** (50 μM) in the presence of increasing concentrations of CT-DNA (0–110 μM). The arrow indicates the absorbance changes upon increasing DNA concentration.

Table 6: Absorbance spectroscopic properties on binding to DNA.

Complex	λ_{max} (nm)		$\Delta\lambda$ (nm)	H (%)	Binding constant (M^{-1})
	Free	Bound			
1	317	318	1	12.40	37.28×10^6
2	322	321	1	35.92	09.75×10^6
3	322	321	1	37.38	13.33×10^6
4	327	329	2	−04.65	12.62×10^6

greater than four, then the exchange interaction is negligible between the copper centers in DMSO medium. This indicates the dissociation of the dimeric complex in polar solvents like DMSO to give mononuclear [Cu(L)Cl], where L is monoanionic thiosemicarbazones. On the other hand, G value less than four indicates the presence of exchange interaction in the solid complex. The ratio of $g_\parallel/A_\parallel$ is used to find the structure of a complex. In present Cu(II) complexes, the ratio obtained is in the range 138–147 cm, which falls in the range 90–150 cm for square-planar copper(II) complexes [40].

Based on the magnetic moments, electronic, infrared, and ESR spectroscopy, the tentative structure of the complexes is shown in Figure 5.

3.4. Binding Characteristics of Complex with DNA. The electronic spectroscopy is used widely to study the binding of the metal complexes with DNA. It has been found that intercalation or electrostatic interaction between the metal complex and DNA leads to hypochromism, whereas hyperchromism indicates the breakdown of the secondary structure of DNA [41]. The absorption titrations have been employed to ascertain the DNA binding strength of complexes by monitoring the changes in the absorption intensity of the ligand-centered bands around 317–322 nm. Figure 6 illustrates the representative absorption spectra for the binding of complexes in absence and presence of CT DNA (at a constant complex concentration, 50 μM). The strong hyperchromism, along with minor red or blue shift for complexes **1**–**3**, indicates strong interaction of the complexes with CT DNA mainly through groove binding [42]. It is known that the hyperchromicity of the UV absorbance band is caused by the unwinding of the double helix as well as its unstacking and the concomitant exposure of the bases, whereas red or blue shift indicates that the complex may have some effect on DNA [42–44]. In case of complex **4**, hypochromism due to $\pi \rightarrow \pi^*$ stacking interaction with a red shift (bathochromism) may appear in the case of an intercalative binding leading to stabilization of DNA duplex [24].

To compare the binding parameters quantitatively, the intrinsic equilibrium binding constant (K_b) for the complexes has been determined, and the higher binding constant values (Table 6) could be due to the presence of aromatic rings, which might facilitate the interaction of the complexes with the DNA bases through noncovalent p-p interaction.

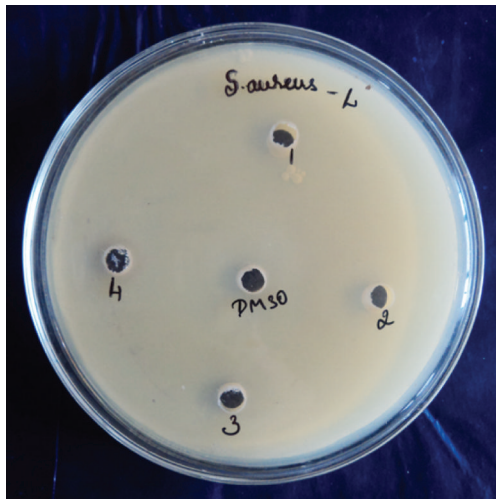
Table 7: Antibacterial and antifungal activities of the compounds.

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Aspergillus niger*	*Alternaria alternate*
$\mathbf{L}_1$	—	+	+	++
1	+	++	—	+
$\mathbf{L}_2$	—	+	—	—
2	—	++	+	+
$\mathbf{L}_3$	—	+	—	+
3	—	+++	—	+
$\mathbf{L}_4$	—	+	—	+
4	+	+	+	+
DMSO	—	—	—	—

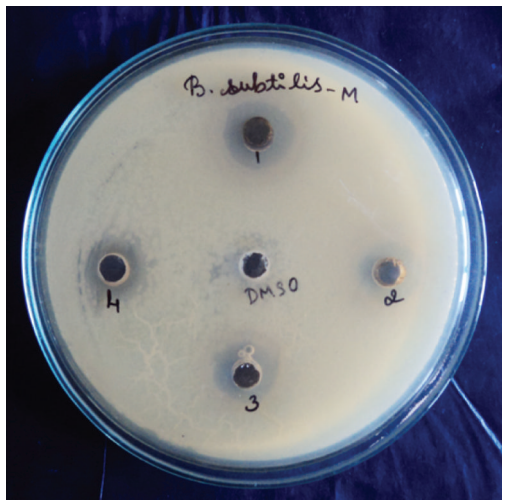
+++ is the activity of zone of clearance radius of 2.5 cm when it is compared to control.
++ is the activity of zone of clearance radius of 1.5 cm when it is compared to control.
+ is the activity of zone of clearance radius of 1.0 and ≤0.6 cm when it is compared to control.
The concentration was 1 mg/mL and prepared in DMSO, and the results are compared with solvent activity also, and each well was loaded with 100 μL, that is, 100 μg was loaded.

3.5. Biological Properties

3.5.1. Antibacterial Activity. The newly synthesized ligands and their complexes were tested for their *in vitro* antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* by using the agar disc diffusion method [25]. Among the tested compounds, three complexes **1**–**3** showed considerable activity almost equal to the activity of ciprofloxacin. The other compounds were found to be moderate or least effective. The compounds have no effect on Gram-negative bacteria whereas they are moderately active for Gram-positive strains. Representative figure of antibacterial activity against *Staphylococcus aureus* (ligands) and *Bacillus subtilis* (metal complexes) is given in Figure 7.



(a)



(b)

Figure 7: *In vitro* antibacterial activity against *Staphylococcus aureus* (ligands) and *Bacillus subtilis* (metal complexes).

3.5.2. Antifungal Activity. The newly synthesized ligands and their complexes were also screened for their antifungal activity against *Aspergillus niger* and *Alternaria alternate* by agar disc diffusion method [26]. The results of the preliminary antifungal testing of the prepared compounds were compared with the typical broad spectrum of the potent antifungal drug amphotericin B. The antifungal activity data (Table 7) reveal that compounds **2**–**4** showed good activity for both the fungal strains whereas $\mathbf{L}_1$ showed excellent activity against *Alternaria alternate*, which is nearly equal to the standard amphotericin B.

4. Conclusions

In summary, four binuclear complexes have been prepared and characterized. The metal ion was coordinated through the thioketonic sulphur and the nitrogen of the azomethine group. The bonding of ligand to metal ions was confirmed by the analytical data, as well as spectral and magnetic studies. The complexes had higher antibacterial and antifungal activities than the ligand. In this study, we have attempted to unravel the DNA interactions of these complexes. The observed trends in binding constants of the complexes may be due to the presence of a phenyl ring in the ligands that facilitate pi-stacking interaction.

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References

[1] I. Pal, F. Basuli, and S. Bhattacharya, "Thiosemicarbazone complexes of the platinum metals. A story of variable coordination modes," *Journal of Chemical Sciences*, vol. 114, no. 4, pp. 255–268, 2002.

[2] M. Belicchi Ferrari, S. Capacchi, G. Pelosi et al., "Synthesis, structural characterization and biological activity of helicin thiosemicarbazone monohydrate and a copper(II) complex of salicylaldehyde thiosemicarbazone," *Inorganica Chimica Acta*, vol. 286, no. 2, pp. 134–141, 1999.

[3] S. Dutta, F. Basuli, S.-M. Peng, G.-H. Lee, and S. Bhattacharya, "Synthesis, structure and redox properties of some thiosemicarbazone complexes of rhodium," *New Journal of Chemistry*, vol. 26, no. 11, pp. 1607–1612, 2002.

[4] A. K. El-Sawaf, D. X. West, F. A. El-Saied, and R. M. El-Bahnasawy, "Synthesis, magnetic and spectral studies of iron(III), cobalt(II,III), nickel(II), copper(II) and zinc(II) complexes of 2-formylpyridine N(4)-antipyrinylthiosemicarbazone," *Transition Metal Chemistry*, vol. 23, no. 5, pp. 649–655, 1998.

[5] S. Purohit, A. P. Koley, L. S. Prasad, P. T. Manoharan, and S. Ghosh, "Chemistry of molybdenum with hard-soft donor ligands. 2. Molybdenum(VI), -(V), and -(IV) oxo complexes with tridentate schiff base ligands," *Inorganic Chemistry*, vol. 28, no. 19, pp. 3735–3742, 1989.

[6] D. X. West, J. K. Swearingen, J. Valdes-Martınez et al., "Spectral and structural studies of iron(III), cobalt(II,III) and nickel(II) complexes of 2-pyridineformamide N(4)-methylthiosemicarbazone," *Polyhedron*, vol. 18, no. 22, pp. 2919–2929, 1999.

[7] A. G. Quiroga, J. M. perez, and I. Lopez-Solera, "Novel tetranuclear orthometalated complexes of Pd(II) and Pt(II) derived from p-isopropylbenzaldehyde thiosemicarbazone with cytotoxic activity in cis-DDP resistant tumor cell lines. Interaction of these complexes with DNA," *Journal of Medicinal Chemistry*, vol. 41, no. 9, pp. 1399–1408, 1998.

[8] R. F. F. Costa, A. P. Rebolledo, T. Matencio et al., "Metal complexes of 2-benzoylpyridine-derived thiosemicarbazones: structural, electrochemical and biological studies," *Journal of Coordination Chemistry*, vol. 58, no. 15, pp. 1307–1319, 2005.

[9] R. K. Agarwal, L. Singh, and D. K. Sharma, "Synthesis, spectral, and biological properties of copper(II) complexes of thiosemicarbazones of schiff bases derived from 4-aminoantipyrine and aromatic aldehydes," *Bioinorganic Chemistry and Applications*, vol. 2006, Article ID 59509, 10 pages, 2006.

[10] O. P. Pandey, S. K. . Sengupta, M. K. Mishra, and C. M. Tripathi, "Synthesis, spectral and antibacterial studies of binuclear titanium(IV) / zirconium(IV) complexes of piperazine dithiosemicarbazones," *Bioinorganic Chemistry and Applications*, vol. 1, no. 1, pp. 35–44, 2003.

[11] C. Shipman Jr., S. H. Smith, J. C. Drach, and D. L. Klayman, "Antiviral activity of 2-acetylpyridine thiosemicarbazones against herpes simplex virus," *Antimicrobial Agents and Chemotherapy*, vol. 19, no. 4, pp. 682–685, 1981.

[12] J. P. Scovill, D. L. Klayman, and C. F. Franchino, "2-acetylpyridine thiosemicarbazones. 4. Complexes with transition metals as antimalarial and antileukemic agents," *Journal of Medicinal Chemistry*, vol. 25, no. 10, pp. 1261–1264, 1982.

[13] P. M. Krishna and K. H. Reddy, "Synthesis, single crystal structure and DNA cleavage studies on first 4N-ethyl substituted three coordinate copper(I) complex of thiosemicarbazone," *Inorganica Chimica Acta*, vol. 362, no. 11, pp. 4185–4190, 2009.

[14] R. G. Zhao, W. Zhang, J. K. Li, and L. Y. Zhang, "(E)-2-Hydroxy-3-methoxy-benzaldehyde thio-semicarbazone," *Acta Crystallographica*, vol. 64, article o1113, 2008.

[15] S. Sen, S. Shit, S. Mitra, and S. R. Batten, "Structural and spectral studies of a new copper(II) complex with a tridentate thiosemicarbazone ligand," *Structural Chemistry*, vol. 19, no. 1, pp. 137–142, 2008.

[16] V. Vrdoljak, I. Dilović, M. Cindrić et al., "Synthesis, structure and properties of eight novel molybdenum(VI) complexes of the types: [MoO2LD] and [MoO2L2D] (L = thiosemicarbazonato ligand, D = N-donor molecule)," *Polyhedron*, vol. 28, no. 5, pp. 959–965, 2009.

[17] V. Vrdoljak, M. Cindrić, D. Matković-Čalogović, B. Prugovečki, P. Novak, and B. Kamenar, "A series of new molybdenum(VI) complexes with the *ONS* donor thiosemicarbazone ligands," *Zeitschrift für Anorganische und Allgemeine Chemie*, vol. 631, no. 5, pp. 928–936, 2005.

[18] V. Vrdoljak, I. Dilović, M. Rubčić et al., "Synthesis and characterisation of thiosemicarbazonato molybdenum(VI) complexes and their *in vitro* antitumor activity," *European Journal of Medicinal Chemistry*, vol. 45, no. 1, pp. 38–48, 2010.

[19] P. Murali Krishna, K. Hussain Reddy, P. G. Krishna, and G. H. Philip, "DNA interactions of mixed ligand copper(II) complexes with sulphur containing ligands," *Indian Journal of Chemistry A*, vol. 46, no. 6, pp. 904–908, 2007.

[20] P. Murali Krishna, K. Hussain Reddy, J. P. Pandey, and D. Siddavattam, "Synthesis, characterization, DNA binding and nuclease activity of binuclear copper(II) complexes of cuminaldehyde thiosemicarbazones," *Transition Metal Chemistry*, vol. 33, no. 5, pp. 661–668, 2008.

[21] P. Murali Krishna, G. N. Anil Kumar, K. Hussain Reddy, and M. K. Kokila, "(2*E*)-*N*-Methyl-2-[(2*E*)-3-phenylprop-2-en-1-ylidene]hydrazinecarbothioamide," *Acta Crystallographica*, vol. E68, article o2842, 2012.

[22] B. S. Shankar, N. Shashidhar, Y. P. Patil, P. Murali Krishna, and M. Nethaji, "2-[(*E*)-2-Hydroxy-3-methoxybenzylidene]-*N*-methylhydrazinecarbothioamide," *Acta Crystallographica*, vol. E69, article o61, 2013.

[23] P. P. T. Sah and T. C. Daniels, "Thiosemicarbazide as a reagent for the identification of aldehydes, ketones, and quinones," *Recueil des Travaux Chimiques des Pays-Bas*, vol. 69, no. 12, pp. 1545–1556, 1950.

[24] A. M. Pyle, J. P. Rehmann, R. Meshoyrer, C. V. Kumar, N. J. Turro, and J. K. Barton, "Mixed-ligand complexes of ruthenium(II): factors governing binding to DNA," *Journal of the American Chemical Society*, vol. 111, no. 8, pp. 3051–3058, 1989.

[25] C. Perez, M. Paul, and P. Bazerque, "An Antibiotic assay by the agar well-diffusion method," *Acta Biologiae et Medicinae Experimentalis*, vol. 15, pp. 113–115, 1990.

[26] R. Nair, T. Kalyariya, and S. Chanda, "Antibacterial activity of some selected Indian medicinal flora," *Turkish Journal of Biology*, vol. 29, pp. 41–47, 2005.

[27] J. P. Phillips and L. L. Merrit, "Ionization constants of some substituted 8-hydroxyquinolines," *Journal of the American Chemical Society*, vol. 70, no. 1, pp. 410–411, 1948.

[28] M. Tumer, H. Koksal, M. K. Sener, and S. Serin, "Antimicrobial activity studies of the binuclear metal complexes derived from tridentate Schiff base ligands," *Transition Metal Chemistry*, vol. 24, no. 4, pp. 414–420, 1999.

[29] P. Bamfield, "The reaction of cobalt halides with N-arylsalicylideneimines," *Journal of the Chemical Society A*, pp. 804–808, 1967.

[30] L. Sacconi, M. Ciampolini, and G. P. Speroni, "Structure mimicry in solid solutions of 3d metal complexes with N-methylsalicylaldimine (Msal-Me)," *Journal of the American Chemical Society*, vol. 87, no. 14, pp. 3102–3106, 1965.

[31] R. P. John, A. Sreekanth, V. Rajakannan, T. A. Ajith, and M. R. P. Kurup, "New copper(II) complexes of 2-hydroxyacetophenone N(4)-substituted thiosemicarbazones and polypyridyl co-ligands: structural, electrochemical and antimicrobial studies," *Polyhedron*, vol. 23, no. 16, pp. 2549–2559, 2004.

[32] M. Joseph, M. Kuriakose, M. R. P. Kurup, E. Suresh, A. Kishore, and S. G. Bhat, "Structural, antimicrobial and spectral studies of copper(II) complexes of 2-benzoylpyridine N(4)-phenyl thiosemicarbazone," *Polyhedron*, vol. 25, no. 1, pp. 61–70, 2006.

[33] K. H. Reddy, P. Sambasiva Reddy, and P. Ravindra Babu, "Synthesis, spectral studies and nuclease activity of mixed ligand copper(II) complexes of heteroaromatic semicarbazones/thiosemicarbazones and pyridine," *Journal of Inorganic Biochemistry*, vol. 77, no. 3-4, pp. 169–176, 1999.

[34] K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds, Part B: Applications in Coordination, Organometallic and BioInorganic Chemistry*, John Wiley & Sons, New York, NY, USA, 5th edition, 1997.

[35] A. C. Hiremath, K. M. Reddy, K. M. Patel, and M. B. Halli, "Metal complexes of Schiff's bases derived from 4-hydrazinobenzofuro [3,2-d]pyrimidines and benzaldehydes," *Proceedings of the National Academy of Sciences, India*, vol. 63, no. 11, pp. 341–345, 1993.

[36] D. N. Sathyanarayana, *Vibrational Spectroscopy*, New Age International, New Delhi, India, 2004.

[37] G. Speie, J. Csihony, A. M. Whalen, and C. G. Pie, "Studies on aerobic reactions of ammonia/3,5-di-tert-butylcatechol schiff-base condensation products with copper, copper(I), and copper(II). Strong copper(II)—radical ferromagnetic exchange and observations on a unique N–N coupling reaction," *Inorganic Chemistry*, vol. 35, pp. 3519–3535, 1996.

[38] R. Neiman and D. Kivelson, "ESR line shapes in glasses of copper complexes," *The Journal of Chemical Physics*, vol. 35, no. 1, pp. 149–155, 1961.

[39] S. M. Mamodoush, S. M. Abou Elenein, and H. M. Kamel, "Study of the critical behavior of polar fluids by renormalization group theory," *Indian Journal of Chemistry A*, vol. 41, pp. 297–303, 2002.

[40] Syamal and R. L. Dutta, *Elements of Magneto Chemistry*, East-West Press, New Delhi, India, 1993.

[41] M. Chauhan, K. Banerjee, and F. Arjmand, "DNA binding studies of novel copper(II) complexes containing L-tryptophan as chiral auxiliary: *in vitro* antitumor activity of Cu-Sn2 complex in human neuroblastoma cells," *Inorganic Chemistry*, vol. 46, no. 8, pp. 3072–3082, 2007.

[42] F. Mancin, P. Scrimin, P. Tecilla, and U. Tonellato, "Artificial metallonucleases," *Chemical Communications*, no. 20, pp. 2540–2548, 2005.

[43] Y. L. Song, Y. T. Li, and Z. Y. Wu, "Synthesis, crystal structure, antibacterial assay and DNA binding activity of new binuclear Cu(II) complexes with bridging oxamidate," *Journal of Inorganic Biochemistry*, vol. 102, no. 9, pp. 1691–1699, 2008.

[44] T. Hirohama, Y. Kuranuki, E. Ebina et al., "Copper(II) complexes of 1,10-phenanthroline-derived ligands: studies on DNA binding properties and nuclease activity," *Journal of Inorganic Biochemistry*, vol. 99, no. 5, pp. 1205–1219, 2005.