

# Potential therapeutic applications of novel metal complexes of (Z)-3-((2-((2-hydroxybenzylidene)amino)phenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one

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**Abstract:** Mono organometallic complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Ag(I) complexes with (Z)-3-((2-((2-hydroxybenzylidene)amino)phenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one ligand has much potential as therapeutic and diagnostic agents. The ligand allows the thermodynamic and kinetic reactivity of the metal ion to be controlled and also provide a scaffold for functionalization. Specific examples involving the design of metal complexes as anticancer agents are discussed. These complexes have been synthesized and characterized by (<sup>1</sup>H-NMR, mass, IR, UV-VIS, ESR) spectra, magnetic moments and conductance measurements, elemental and thermal analyses (TGA and DTA). Molar conductances in DMF solution indicates that, the complexes are non-electrolytes. The ESR spectra of solid Cu(II) complexes (2-4) show an anisotropic or isotropic type indicating a  $d(x^2-y^2)$  ground state with a significant covalent bond character. However, Mn(II) complex (5) and Co(II) Complex (8) show an isotropic type indicating an octahedral geometry. Cytotoxic evolution  $IC_{50}$  of the ligand and its complexes have been carried out. Zn(II) complex (7) and Ag(I) complex (9) show enhanced activity in comparison to the parent ligand or standard drug. Cu(II) complexes (2) and (3) have been done. Copper is enriched in various human cancer tissues and is a co-factor essential for tumor angiogenesis processes. However, the use of copper binding ligand to target tumor, copper could provide a novel strategy for cancer selective treatment. Also, antimicrobial studies of the ligand and some of its complexes are described.

**Keywords:** Complexes, Synthesis, Schiff Base, ESR, Cytotoxicity.

## 1 INTRODUCTION

Schiff bases have assumed a key part in the advancement of coordination science. Schiff-base mixes containing an imine gather are generally framed by the buildup of an essential amines with a dynamic carbonyl or aldehyde gathering. These mixes are considered as an essential class of natural mixes, which have wide applications in numerous organic perspectives [1]. Various Schiff- bases buildings were accounted for to have geno-lethality [2,3], antibacterial [4,5] and antifungal exercises [6]. The expanding enthusiasm for progress metal edifices containing Schiff-base ligand is gotten from their settled part in natural frameworks and in addition their reactant and pharmaceutical application [7]. Metal edifices give an exceptionally flexible stage to sedate plan. Other than variety in the metal and its oxidation express, that permit the calibrating of their concoction reactivity regarding both energy and thermodynamics. The metal as well as the ligands can assume imperative parts in natural action, extending from external circle acknowledgment of the objective site to the movement of any discharged ligands and ligand focused redox forms. Because

of a developing enthusiasm for the in the improvement of metallo-helpful medications and metal-based operators [8,9], we revealed in this amalgamation and portrayal of new metallo-helpful applicants got from the novel ligand (Z)-3-((2-((2-hydroxybenzylidene)amino)phenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one. The cytotoxic movement of blended mixes has been likewise explored.

## 2 MATERIALS AND METHODS

All the reagents employed for the preparation of the ligand and its complexes were synthetic grade and used without further purification. TLC is used to confirm the purity of the compounds. C, H, N and Cl analyses were determined at the Analytical Unit of Cairo University, Egypt. A standard gravimetric method was used to determine metal ions [10,12]. All metal complexes were dried under vacuum over  $P_4O_{10}$ . The IR spectra were measured as KBr pellets using a Perkin-Elmer 683 spectrophotometer ( $4000-400\text{ cm}^{-1}$ ). Elec-

tronic spectra (qualitative) were recorded on a Perkin-Elmer 550 spectrophotometer. The conductance ( $10^{-3}M$ ) of the complexes in DMF were measured at 25 °C with a Bibby conduct meter type MCI.  $^1H$ -NMR spectra of the ligand and its Zn(II) complex (**7**) were obtained with Perkin-Elmer R32-90-MHz spectrophotometer using TMS as internal standard. Mass spectra were recorded using JEULJMS-AX-500 mass spectrometer provided with data system. The thermal analyses (DTA and TGA) were carried out in air on a Shimadzu DT-30 thermal analyzer from 27 to 800 °C at a heating rate of 10 °C per minute. Magnetic susceptibilities were measured at 25 °C by the Gouy method using mercuric tetrathiocyanatocobalt(II) as the magnetic susceptibility standard. Diamagnetic corrections were estimated from Pascal's constant [13]. The magnetic moments were calculated from the equation: ( $\mu_{eff} = 2.828 (X_n \times T)^{1/2}$ ) (1)

The ESR spectra of solid complexes at room temperature were recorded using a varian E-109 spectrophotometer, DPPH was used as a standard material. The TLC of all compounds confirmed their purity.

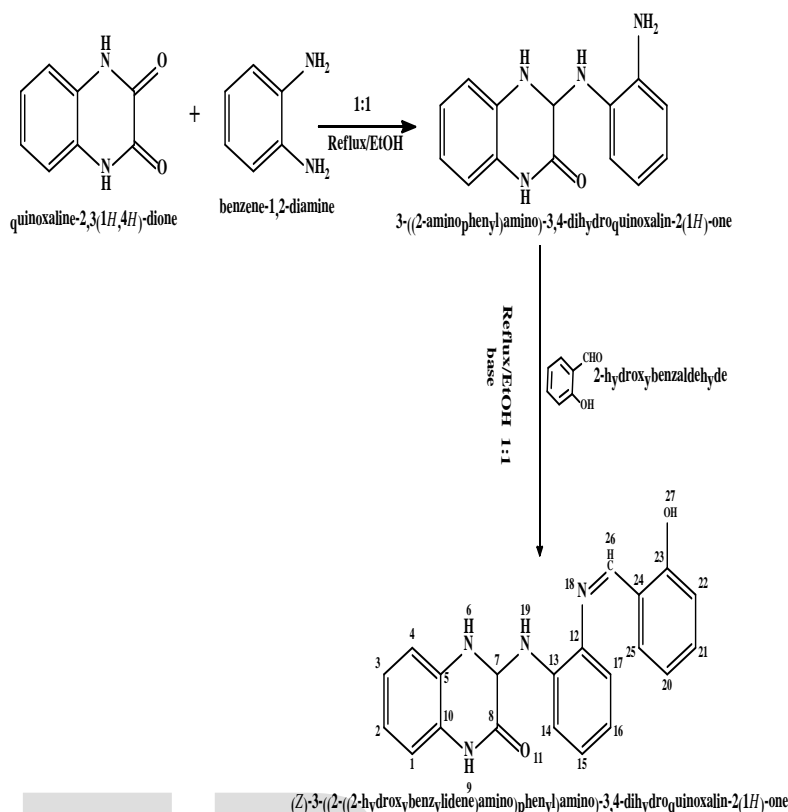
## 2.1 PREPARATION OF THE LIGAND

### 2.1.1 PREPARATION OF 3-((2-AMINOPHENYL)AMINO)-3,4-DIHYDROQUINOXALIN-2(1H)-ONE:

3-((2-aminophenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (Scheme 1) was prepared by adding equimolar amount of quinoxaline-2,3(1H,4H)-dione (15.70 g, 1 mol), to benzene-1,2-diamine (10.49 g, 1 mol) in 50 cm<sup>3</sup> of absolute ethanol. The mixture was refluxed with stirring on water bath for 2 hours and then left to cool at room temperature, filtered off, washed with water, dried and recrystallized from ethanol to afford 3-((2-aminophenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one.

### 2.1.2 PREPARATION OF THE SCHIFF-BASE LIGAND (Z)-3-((2-((2-HYDROXYBENZYLIDENE)AMINO)PHENYL)AMINO)-3,4-DIHYDROQUINOXALIN-2(1H)-ONE (HL):

The ligand [HL] (Z)-3-((2-((2-hydroxybenzylidene)amino)phenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (Scheme 1) was prepared by adding equimolar amount of 3-((2-aminophenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one (22.40 g, 1 mol) to salicylaldehyde (11.33 g, 1mol) in 50 cm<sup>3</sup> of absolute ethanol. The mixture was refluxed with stirring on water bath for 2 hours and then left to cool at room temperature, filtered off, washed with water, dried and recrystallized from ethanol to afford ligand (**1**). Ligand (**1**): Yield 91 %; m.p. 297; color is brown; Anal. Calcd. (%) for C<sub>21</sub>H<sub>26</sub>N<sub>4</sub>O<sub>7</sub> (FW = 446.45): C, 56.50; H, 5.87; N, 12.55; Found (%) C, 57.1; H, 5.91; N, 12.60; IR (KBr, cm<sup>-1</sup>), 3450,  $\nu$ (OH), 3260m,  $\nu$ (NH), 1622  $\nu$ (C=N), 1477, 758  $\nu$ (C=C)<sub>Ar</sub>, 1615  $\nu$ (C=C)<sub>Al</sub>, 1324  $\nu$ (C-OH), 1040  $\nu$ (N-N);  $^1H$ -NMR [DMSO-*d*<sub>6</sub>]: 10 (s, 1H, <sup>26</sup>OH); 8.8 (s, 1H, NH<sup>7</sup>); 8.7 (s, 1H, NH<sup>11</sup>); 8.40 (s, 1H, <sup>19</sup>H-C=N); 9.1 (s, 1H, NH<sup>10</sup>); 3.4 (s, 1H, H<sup>8</sup>); 6.04-7.8 (m, 10 H, aromatic protons).



**Scheme 1:** Preparation of Ligand, (Z)-3-((2-((2-hydroxybenzylidene)amino)phenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one

## 2.2 SYNTHESIS OF METAL COMPLEXES (2)-(9)

The metal complexes **2-9** were prepared by refluxing with string a suitable amount (1 mmol) of a hot ethanolic solution of the following metal salts: Cu(CH<sub>3</sub>COO)<sub>2</sub>·H<sub>2</sub>O, CuCl<sub>2</sub>·2H<sub>2</sub>O, CuSO<sub>4</sub>·5H<sub>2</sub>O, Mn(CH<sub>3</sub>COO)<sub>2</sub>·4H<sub>2</sub>O, Ni(CH<sub>3</sub>COO)<sub>2</sub>·4H<sub>2</sub>O, Zn(CH<sub>3</sub>COO)<sub>2</sub>·2H<sub>2</sub>O, Co(CH<sub>3</sub>COO)<sub>2</sub>·4H<sub>2</sub>O and Ag(CH<sub>3</sub>COO) (1 mmol) with a hot ethanolic solution of the ligand (4.0 g 1 mmol, 40 mL ethanol). The refluxing times varied from 2 to 6 hours according to the depending to nature of metal ion, which led to precipitate of metal complexes. The precipitates, were filtered off, washed with ethanol then by diethyl ether and dried in vacuum desiccators over P<sub>4</sub>O<sub>10</sub>. Analytical data for the prepared complexes are: **Complex (2)**, [(L)Cu(CH<sub>3</sub>COO)(H<sub>2</sub>O)<sub>2</sub>].3H<sub>2</sub>O: Yield: 85%; m.p. >300 °C; color: black; molar conductivity ( $\Lambda_m$ ): 7.8 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>. Anal. Calcd. (%) for C<sub>23</sub>H<sub>28</sub>N<sub>4</sub>O<sub>9</sub>Cu (FW = 568.04): C, 48.63; H, 4.97; N, 9.86, Cu, 11.19; Found (%) C, 49.2; H, 5.21; N, 9.99, Cu, 11.31; IR (KBr, cm<sup>-1</sup>), 3100  $\nu$ (NH), 1600  $\nu$ (HC=N), 1550  $\nu$ (C=N), 1668  $\nu$ (C=O), 1319  $\nu$ (C-O), 609  $\nu$ (M←O), 502  $\nu$ (M←N), 1530, 1370  $\nu_{sym}$ CH<sub>3</sub>COO,  $\nu_{asym}$ CH<sub>3</sub>COO ( $\Delta$ =160 cm<sup>-1</sup>).

**Complex (3)**, [(HL)(Cu)Cl<sub>2</sub>(H<sub>2</sub>O)].2H<sub>2</sub>O: Yield: 80 %; m.p. >300 °C; color: black; molar conductivity ( $\Lambda_m$ ): 9.2 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>. Anal. Calcd. (%) for C<sub>21</sub>H<sub>22</sub>N<sub>4</sub>O<sub>5</sub>Cl<sub>2</sub>Cu (FW = 544.88): C, 46.29; H, 4.07; N, 10.28, Cu, 11.66; Found (%) C, 46.40; H, 4.10; N, 10.32, Cu, 11.74; IR (KBr, cm<sup>-1</sup>), 3403  $\nu$ (OH), 3171  $\nu$ (NH), 1550  $\nu$ (HC=N), 1533  $\nu$ (C=N), 1683  $\nu$ (C=O), 1406  $\nu$ (C-O), 584  $\nu$ (M←O), 509  $\nu$ (M←N), 459  $\nu$ (M-Cl).

**Complex (4)**, [(HL)Cu(SO<sub>4</sub>)(H<sub>2</sub>O)<sub>2</sub>].2H<sub>2</sub>O: Yield: 79 %; m.p. >300 °C; color: brown; molar conductivity ( $\Lambda_m$ ): 9.2 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>. Anal. Calcd. (%) for C<sub>21</sub>H<sub>24</sub>N<sub>4</sub>O<sub>10</sub>SCu (FW = 588.05): C, 42.89; H, 4.11; N, 9.53, Cu, 10.81; Found (%) C, 43.01; H, 4.21; N, 9.71, Cu, 10.99; IR (KBr, cm<sup>-1</sup>), 3379  $\nu$ (OH), 3200  $\nu$ (NH), 1626  $\nu$ (HC=N), 1530  $\nu$ (C=N), 1680  $\nu$ (C=O), 1316  $\nu$ (C-O), 615  $\nu$ (M←O), 510  $\nu$ (M←N), 1197, 1149, 1094, 995, 864, 758  $\nu$ (SO<sub>4</sub>).

**Complex (5)**, [(HL)Mn(CH<sub>3</sub>COO)<sub>2</sub>(H<sub>2</sub>O)].2H<sub>2</sub>O: Yield: 67 %; m.p. >300 °C; color: brown; molar conductivity ( $\Lambda_m$ ): 9.7 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>. Anal. Calcd. (%) for C<sub>25</sub>H<sub>28</sub>N<sub>4</sub>O<sub>9</sub>Mn (FW = 583.45): C, 51.46; H, 4.84; N, 9.60, Mn, 9.42; Found (%) C, 51.61; H, 4.91; N, 9.71, Mn, 9.62; IR (KBr, cm<sup>-1</sup>), 3385  $\nu$ (OH), 3180  $\nu$ (NH), 1601  $\nu$ (HC=N), 1540  $\nu$ (C=N), 1679  $\nu$ (C=O), 1313  $\nu$ (C-O), 616  $\nu$ (M←O), 495  $\nu$ (M←N), 1530, 1253  $\nu_{\text{sym}}$ CH<sub>3</sub>COO,  $\nu_{\text{asym}}$ CH<sub>3</sub>COO ( $\Delta=277$  cm<sup>-1</sup>).

**Complex (6)**, [(HL)Ni(CH<sub>3</sub>COO)<sub>2</sub>(H<sub>2</sub>O)].2H<sub>2</sub>O: Yield: 74 %; m.p. >300 °C; color: black; molar conductivity ( $\Lambda_m$ ): 6.12 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>. Anal. Calcd. (%) for C<sub>25</sub>H<sub>28</sub>N<sub>4</sub>O<sub>9</sub>Ni (FW = 587.20): C, 51.14; H, 4.81; N, 9.54, Ni, 10.00; Found (%) C, 51.21; H, 4.93; N, 9.71, Ni, 10.00; IR (KBr, cm<sup>-1</sup>), 3407  $\nu$ (OH), 3030  $\nu$ (NH), 1603  $\nu$ (HC=N), 1531  $\nu$ (C=N), 1671  $\nu$ (C=O), 1310  $\nu$ (C-O), 607  $\nu$ (M←O), 499  $\nu$ (M←N), 1519, 1371  $\nu_{\text{sym}}$ CH<sub>3</sub>COO,  $\nu_{\text{asym}}$ CH<sub>3</sub>COO ( $\Delta=148$  cm<sup>-1</sup>).

**Complex (7)**, [(HL)Zn(CH<sub>3</sub>COO)<sub>2</sub>(H<sub>2</sub>O)].2H<sub>2</sub>O: Yield: 85 %; m.p. >300 °C; color: brown; molar conductivity ( $\Lambda_m$ ): 7.9 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>. For Anal. Calcd. (%) for C<sub>25</sub>H<sub>28</sub>N<sub>4</sub>O<sub>9</sub>Zn (FW = 593.89): C, 50.56; H, 4.75; N, 9.43 Zn, 11.01; Found (%) C, 50.63; H, 4.86; N, 9.64, Zn, 11.21; IR (KBr, cm<sup>-1</sup>), 3385  $\nu$ (OH), 3150  $\nu$ (NH), 1602  $\nu$ (HC=N), 1540  $\nu$ (C=N), 1667  $\nu$ (C=O), 1312  $\nu$ (C-O), 617  $\nu$ (M←O), 500  $\nu$ (M←N), 1531, 1407  $\nu_{\text{sym}}$ CH<sub>3</sub>COO,  $\nu_{\text{asym}}$ CH<sub>3</sub>COO ( $\Delta=124$  cm<sup>-1</sup>).

**Complex (8)**, [(HL)Co(SO<sub>4</sub>)(H<sub>2</sub>O)<sub>2</sub>].2H<sub>2</sub>O: Yield: 80 %; m.p. =194 °C; color: green; molar conductivity ( $\Lambda_m$ ): 7.5 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>. For Anal. Calcd. (%) for C<sub>21</sub>H<sub>24</sub>N<sub>4</sub>O<sub>10</sub>Co (FW = 583.43): C, 43.23; H, 4.15; N, 9.60, Co, 10.10; Found (%) C, 43.32; H, 4.23; N, 9.64, Co, 10.31; IR (KBr, cm<sup>-1</sup>), 3375  $\nu$ (OH), 3050  $\nu$ (NH), 1624  $\nu$ (HC=N), 1550  $\nu$ (C=N), 1670  $\nu$ (C=O), 1313  $\nu$ (C-O), 604  $\nu$ (M←O), 492  $\nu$ (M←N), 1190, 1149, 1093, 990,860,829  $\nu$ (SO<sub>4</sub>).

**Complex (9)**, [(HL)Ag(CH<sub>3</sub>COO)] .2H<sub>2</sub>O: Yield: 69%; m.p.>300 °C; color: reddish brown; molar conductivity ( $\Lambda_m$ ): 9.8 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>. Anal. Calcd. (%) for C<sub>23</sub>H<sub>23</sub>N<sub>4</sub>O<sub>6</sub>Ag (FW = 559.32): C, 49.39; H, 4.14; N, 10.02, Ag, 19.29; Found (%) C, 49.43; H, 4.21; N, 10.24,

Ag, 19.32; IR (KBr, cm<sup>-1</sup>), 3408  $\nu$ (OH), 3180  $\nu$ (NH), 1602  $\nu$ (HC=N), 1550  $\nu$ (C=N), 1672  $\nu$ (C=O), 1315  $\nu$ (C-O), 617  $\nu$ (M←O), 501  $\nu$ (M←N), 1529, 1458  $\nu_{\text{sym}}$ CH<sub>3</sub>COO,  $\nu_{\text{asym}}$ CH<sub>3</sub>COO ( $\Delta=71$  cm<sup>-1</sup>).

## 2.3 BIOLOGICAL ACTIVITY

**Cytotoxic activity:** Evaluation of the cytotoxic activity of the ligand and its metal complexes was carried out in the Pathology Laboratory, Pathology Department, Faculty of Medicine, El-Menoufia University, Egypt. The evaluation process was carried out in vitro using the Sulfo-Rhodamine-B-stain (SRB) assay published method [14,15]. Cells were plated in 96-multiwell plate (104cells/well) for 24 hrs. Before treatment with the complexes to allow attachment of cell to the wall of the plate. Different concentrations of the compounds under test in DMSO (0, 5, 12.5, 25 and 50  $\mu$ g/ml) were added to the cell monolayer, triplicate wells being prepared for each individual dose. Monolayer cells were incubated with the complexes for 48 hrs.at 37°C and under 5% CO<sub>2</sub>. After 48 hrs. cells were fixed, washed and stained with Sulfo-Rhodamine-B-stain. Excess stain was wash with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each tumor cell line after addition the specified compound.

## 3 RESULTS AND DISCUSSION

All the metal complexes are stable at room temperature, non hydroscopic, insoluble in water, partially soluble in MeOH, EtOH, CHCl<sub>3</sub> and (CH<sub>3</sub>)<sub>2</sub>CO and completely soluble in DMF and DMSO. The analytical and physical data, spectral data (experimental part, Tables 1-2) are compatible with the proposed structures, Figure 1-4. The molar conductance of the complexes in 10<sup>-3</sup> M DMF at 25 °C are in the 10.12-4.72 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup> range, indicating a non-electrolytic nature [19]. These low values commensurate the absence of any counter ions in their structure [17]. Many attempts were made to grow a single crystal but unfortunately, they were failed. Reaction of the ligand (1) with metal salts using (1L: 1M) molar ratio in ethanol gives complexes (2)-(9).

### 3.1 PROTON NUCLEAR MAGNETIC RESONANCE SPECTRA (1H-NMR ) OF THE LIGAND (1) AND Zn(II) COMPLEX (7)

The <sup>1</sup>H-NMR spectra of ligand and Zn(II) complex (7) in deuterated DMSO show peaks consistent with the proposed structure. The

<sup>1</sup>H-NMR spectrum of the ligand shows chemical shift observed as singlet at 13.8 ppm (s, H, OH<sup>27</sup>) which is assigned to proton of aromatic hydroxyl group. The chemical shifts which appeared at 8.1-8.3 ppm range is attributed to the azomethine protons (<sup>26</sup>H-C=N). However, the chemical shifts appeared as a singlet at 5.4 ppm is attributed to the proton of NH group. A set of signals appeared as multiples in the 6.2-7.9 ppm range, corresponding to protons of aromatic ring [21]. By comparison the <sup>1</sup>H NMR of the ligand and the spectrum of the Zn(II) complex (7). The presence of the signal shifted to downfield shift characteristic to the OH group indicating that the ligand bonded with the Zn(II) ions in its protonated form. In addition, there is a significant downfield shift of the azomethine proton signal and one from NH groups relative to the free ligand clarified that the metal ions are coordinated to the azomethine nitrogen atom and NH nitrogen atom. This shift may be due to the formation of a coordination bond (N→M) [19,17].

### 3.2 MASS SPECTRA

The mass spectra of (1), Ni(II) complex (6) and Zn(II) complex (7) confirmed their proposed formulation. The spectrum of (1) reveals the molecular ion peak (m/z) at 446.45 amu consistent with the molecular weight of the ligand. Furthermore, the fragments observed at (m/z) = 52, 77, 182, 210, 356 and 446 amu correspond to C<sub>4</sub>H<sub>4</sub>, C<sub>6</sub>H<sub>5</sub>, C<sub>14</sub>H<sub>14</sub>, C<sub>16</sub>H<sub>18</sub>, C<sub>21</sub>H<sub>16</sub>N<sub>4</sub>O<sub>2</sub> and C<sub>21</sub>H<sub>26</sub>N<sub>4</sub>O<sub>7</sub> moieties respectively. Complex (6) shows fragments (m/z) at 55, 108, 270, 494 and 587 amu due to C<sub>4</sub>H<sub>7</sub>, C<sub>8</sub>H<sub>12</sub>, C<sub>18</sub>H<sub>26</sub>N<sub>2</sub>, C<sub>22</sub>H<sub>19</sub>N<sub>4</sub>NiO<sub>6</sub> and C<sub>25</sub>H<sub>28</sub>N<sub>4</sub>NiO<sub>9</sub> moieties respectively. The fragments observed (m/z) at 59, 93, 299, 355 and 593 amu for complex (7) were assigned to C<sub>4</sub>H<sub>11</sub>, C<sub>7</sub>H<sub>9</sub>, C<sub>19</sub>H<sub>15</sub>N<sub>4</sub>, C<sub>21</sub>H<sub>15</sub>N<sub>4</sub>O<sub>2</sub> and C<sub>25</sub>H<sub>28</sub>N<sub>4</sub>ZnO<sub>9</sub> moieties.

### 3.3 INFRARED SPECTRA (IR)

The mode of bonding between the ligand and the metal ion revealed by comparing the IR spectra of the ligand (1) and its metal complexes (2)-(9). The ligand shows bands in the 3610-3230 and 3220-2500 cm<sup>-1</sup> ranges, commensurate the presence of two types of intra- and intermolecular hydrogen bonds of OH and NH groups with imine group [24]. Thus, the higher frequency band is associated with a weaker hydrogen bond. The medium band at 3260 cm<sup>-1</sup> is assigned to ν(NH) groups [24,25]. The ν(NH) group in the complexes appears shifted from the region of the free ligand indicating that, the NH group is involved in the coordination to the metal ion [26]. However, the characteristic bands of imines, ν(C=N) and ν(CH=C) were observed at 1623 and 1615 cm<sup>-1</sup> respectively. Strong band appears at 1393 cm<sup>-1</sup> is attributed to the ν(C-OH) vibration. The bands appear at 1490 and 762 cm<sup>-1</sup> range, are assigned to ν(Ar) vibration [26,27]. By comparing the IR spectra of the complexes (2)-(9) with that of the free ligand. It was found that, the position of the ν(C=N) bands of imines is shifted by 2-72 cm<sup>-1</sup> range towards lower wavenumber in the complexes indicating coordination through nitrogen of azomethine group (CH=N) [26,27]. This is also confirmed by the appearance of new bands in the 510-492 cm<sup>-1</sup> range, this has been

assigned to the ν(M-N) [27]. Complexes (2) shows ν(C-O) band at 1319 cm<sup>-1</sup>. However, complexes (3)-(9) show ν(C-OH) in the 1406-1310 range, indicating coordination to the metal ion<sup>1</sup> indicating protonated of (C-OH) and lowering the value of the group confirming coordinated to the metal ion [28]. The aromatic ring to the metal ion appears in the 1480-1455 cm<sup>-1</sup> and 754-747 cm<sup>-1</sup> ranges [30]. The IR spectra of the metal complexes (2)-(9) show bands in the 3630-3521 cm<sup>-1</sup>, 3700-3201 cm<sup>-1</sup>, 3280-3210 cm<sup>-1</sup> and 2760-2470 cm<sup>-1</sup> ranges, commensurate the presence of two types of intra- and intermolecular hydrogen bonds. In acetate complexes, the acetate ion may be coordinate to the metal ion in unidentate manner [29]. In the case of acetate complexes (2), (5), (6), (7) and (9) show bands in the 1253-1458 and 1519-1529 cm<sup>-1</sup> ranges, assigned to the asymmetric and symmetric stretches of the COO group. The mode of coordination of acetate group has often been deduced from the magnitude of the observed separation between the ν<sub>asym</sub>(COO<sup>-</sup>) and ν<sub>sym</sub>(COO<sup>-</sup>). The separation value (Δ) between ν<sub>asym</sub>(COO<sup>-</sup>) and ν<sub>sym</sub>(COO<sup>-</sup>) in this complex was in the 71-277 cm<sup>-1</sup> range suggesting the coordination of acetate group in these complexes as a monodentate fashion [24,29]. The sulphato complex (4) shows bands at 1197, 1149, 1094, 995, 864 and 758 cm<sup>-1</sup>, and complex (8) shows bands at 1190, 1149, 1093, 990, 860 and 829 cm<sup>-1</sup> which assigned to monodentate sulphate group [30]. Complexes (2)-(9) show bands in the 510-492 cm<sup>-1</sup> is assigned to ν(M-N) [29]. Complexes (2)-(9) show bands in the 617-584 cm<sup>-1</sup> are due to ν(M-O) [28].

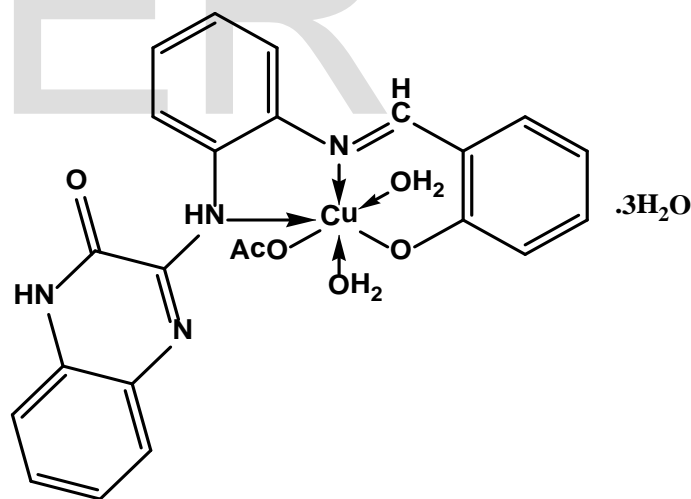


Fig. 1: Structure representation of Cu(II) complex (2)

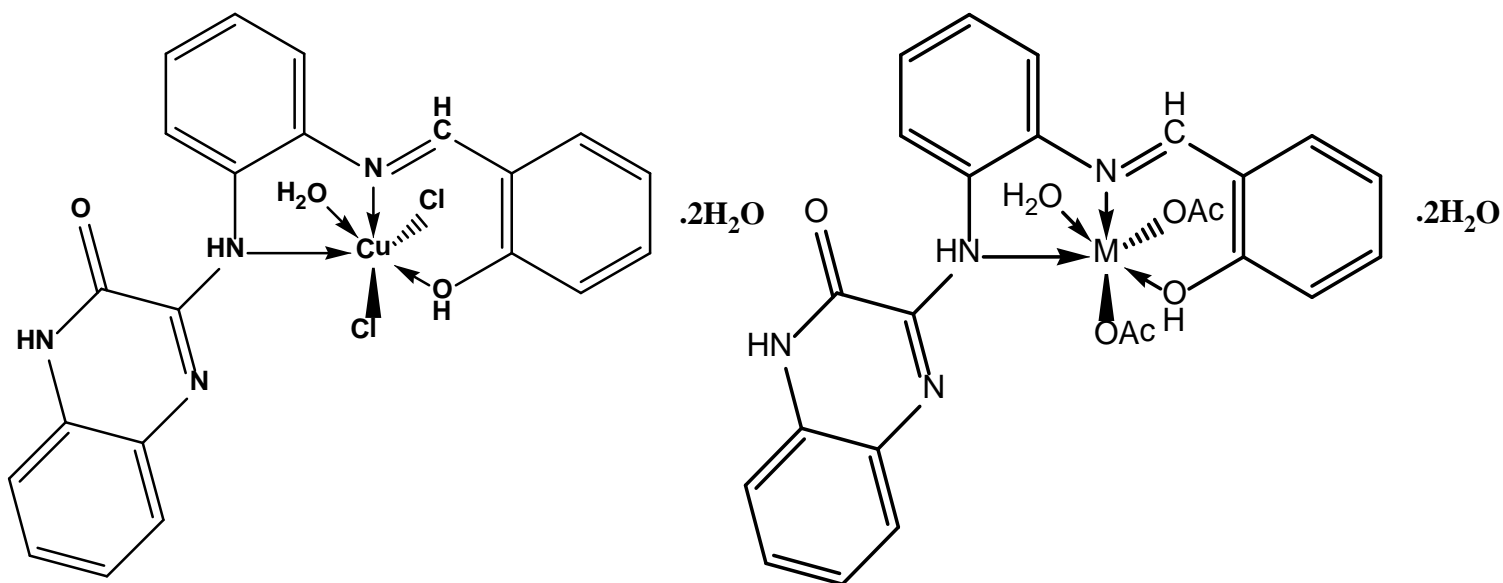


Fig. 2: Structure representation of Cu(II) complex (3)

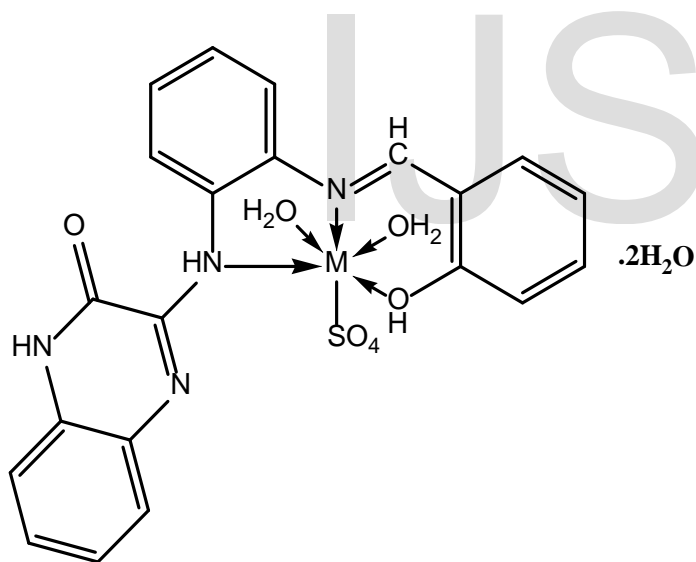
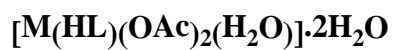


Fig. 4: Structure representation of Mn(II), Ni(II) and Zn(II) complexes (5), (6) and (7)

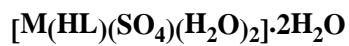


Fig. 3: Structure representation of Cu(II), Co(II) complexes (4) and (8)

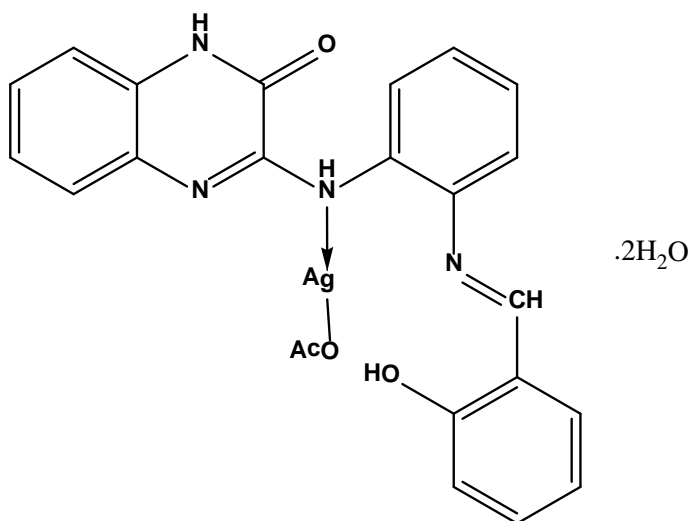


Fig. 5: Structure representation of Ag(I) complex (9)

### 3.4 Magnetic moments

The magnetic moments of the metal complexes (2)-(9) at room temperatures are shown in (Table 1). Copper(II) complexes (2)-(4) show values in the 1.69-1.71 B.M, range corresponding to one unpaired electron in an octahedral structure and these low value indicates spin-spin interactions takes place between Cu(II) ion [17,32]. Manganese(II) complex (5) show value 5.73, indicating high spin octahedral geometry around the Mn(II) ion [24,34]. Zn(II) complexes (7) and Ag(I) Complex (9) show diamagnetic property [35]. Nickel(II) complex (6) shows value 3.12 B.M, indicating high spin octahedral geometry around the Ni(II) ion and cobalt(II) complex (8) shows value 4.75 B.M, high spin octahedral cobalt(II) complex [36].

### 3.5 Electronic spectra

The electronic spectral data for the ligand (1) and its metal complexes in DMF solution are summarized in (Table 1). Ligand (1) in DMF solution shows two bands at 320 nm ( $\epsilon = 7.72 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) and 295 nm ( $\epsilon = 7.12 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) which may be assigned to  $n \rightarrow \pi^*$  and  $\pi \rightarrow \pi^*$  transitions of the imine and aromatic ring respectively [33]. Copper(II) complexes (2), (3), and (4) show bands in the 295-275 and 310-305 nm ranges, these bands are due to intraligand transitions, however, the bands appear in the 495-450, 580-500 and 620-600 nm ranges, are assigned to  $O \rightarrow Cu$ , charge transfer,  ${}^2B_1 \rightarrow {}^2E$  and  ${}^2B_1 \rightarrow {}^2B_2$  transitions, indicating a distorted tetragonal octahedral structure [37,38]. However, manganese(II) complex (5) show bands in the 288-285, 340-300, 440-400, 585-510 and 623-620 nm, the first two bands are within the ligand, however, the other bands are corresponding to  ${}^6A_{1g} \rightarrow {}^4E_g$ ,  ${}^6A_{1g} \rightarrow {}^4T_{2g}$  and  ${}^6A_{1g} \rightarrow {}^4T_{1g}$  transitions which are compatible to an octahedral geometry around the Mn(II) ion [42]. Nickel(II) complex (6) shows bands in the 280-305, 450, 620 and 765 nm, the first bands are within the ligand and the other bands are attributable to  $O \rightarrow Ni$  charge transfer,  ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)(v_3)$ ,  ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)(v_2)$  and  ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)(v_1)$  transitions respectively, indicating an octahedral Ni(II) geometry [37]. The  $v_2/v_1$  ratio for

(6) is 1.3 which is less than the usual range of 1.5-1.75, indicating a distorted octahedral Ni(II) complex. However, cobalt(II) complex (8) shows bands in the 285-310 nm range are within the ligand and 440, 510 and 620 nm are assigned to  ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)(v_3)$ ,  ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(v_2)$  and  ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)(v_1)$  transitions [37]. Zinc(II) complex (7) and silver(I) complex (10) show bands due to intraligand transitions [42].

Table 1: Electronic Spectra (nm) and magnetic moments (B.M) for the Ligand and Its Complexes

No.	Ligand/Complexes	$\lambda_{max}$ (nm)	$\mu_{eff}$ (B.M)	$v_2/v_1$
(1)	[HL]	295 nm ( $\epsilon = 7.12 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$ ) 320 nm ( $\epsilon = 7.72 \times 10^3 \text{ mol}^{-1} \text{ cm}^{-1}$ )	-	-
(2)	[(L)Cu(CH <sub>3</sub> COO)(H <sub>2</sub> O) <sub>2</sub> ].3H <sub>2</sub> O	290,310,495,540,600	1.70	-
(3)	[(HL)CuCl <sub>2</sub> (H <sub>2</sub> O)].2H <sub>2</sub> O	285,293,310,465,580,610	1.69	-
(4)	[(HL)Cu(SO <sub>4</sub> )(H <sub>2</sub> O) <sub>2</sub> ].2H <sub>2</sub> O	275,305, 450,500,600,620	1.71	-
(5)	[(HL)Mn(CH <sub>3</sub> COO) <sub>2</sub> (H <sub>2</sub> O)].2H <sub>2</sub> O	285,288,300,340,400, 440,510,585,620, 623	5.73	-
(6)	[(HL)Ni(CH <sub>3</sub> COO) <sub>2</sub> (H <sub>2</sub> O)].2H <sub>2</sub> O	280,295,305,307,450,565,620,765	3.12	1.3
(7)	[(HL)Zn(CH <sub>3</sub> COO) <sub>2</sub> (H <sub>2</sub> O)].2H <sub>2</sub> O	290,300,455,565,615	Dia.	--
(8)	[(HL)Co(SO <sub>4</sub> )(H <sub>2</sub> O) <sub>2</sub> ].2H <sub>2</sub> O	285,300,340,400, 440,510,585,620, 623	4.75	-
(9)	[(HL)Ag(CH <sub>3</sub> COO)].2H <sub>2</sub> O	285,288,305,330,420,620	Dia.	-

### 3.6 Electron spin resonance (ESR)

The ESR spectral data for complexes (2)-(4), Mn(II) complex (5) and Co(II) complex (8) are presented in (Table 2a). The spectra of copper(II) complexes (2-3) are characteristic of species  $d^9$  configuration having axial type of a  $d_{(x^2-y^2)}$  ground state which is the most common for copper(II) complexes [25,29]. The complexes show  $g_{\parallel} > g_{\perp} > 2.0023$ , indicating octahedral geometry around copper(II) ion [45,46]. The g-values are related by the expression  $G = (g_{\parallel} - 2) / (g_{\perp} - 2)$  [45,47], where (G) exchange coupling interaction parameter (G). If  $G < 4.0$ , a significant exchange coupling is present, whereas if  $G > 4.0$ , local tetragonal axes are aligned parallel or only slightly misaligned. Complexes (2) and (3) show 2.4 and 2.5 values indicating spin-exchange interactions take place between copper(II) ions. This phenomena is further confirmed by the magnetic moments values at (1.69 and 1.70 B.M). The  $g_{\parallel}/A_{\parallel}$  value is also considered as a diagnostic term for stereochemistry [48], the  $g_{\parallel}/A_{\parallel}$  values in the (183-222  $\text{cm}^{-1}$ ) range are expected for copper complexes within perfectly square planar geometry and for tetragonal distorted octahedral complexes are 150-250  $\text{cm}^{-1}$ . The  $g_{\parallel}/A_{\parallel}$  values for the copper complexes are lie just within the range expected for the tetragonal distorted octahedral copper(II) complexes (Table 2a). The g-value of the copper(II) complexes with a  ${}^2B_{1g}$  ground state ( $g_{\parallel} > g_{\perp}$ ) may be expressed by [49]:

$$g_{\parallel} = 2.002 - (8K^2_{\parallel} \lambda^{\circ} / \Delta E_{xy}) \quad (2)$$

$$g_{\perp} = 2.002 - (2K^2_{\perp} \lambda^{\circ} / \Delta E_{xz}) \quad (3)$$

Where  $k_{\parallel}$  and  $k_{\perp}$  are the parallel and perpendicular components respectively of the orbital reduction factor (K),  $\lambda^{\circ}$  is the spin-orbit

coupling constant for the free copper,  $\Delta E_{xy}$  and  $\Delta E_{xz}$  are the electron transition energies of  ${}^2B_{1g} \rightarrow {}^2B_{2g}$  and  ${}^2B_{1g} \rightarrow {}^2E_g$ . From the above relations, the orbital reduction factors ( $K_{||}$ ,  $K_{\perp}$ ,  $K$ ), which are measure terms for covalency [58], can be calculated. For an ionic environment,  $K=1$ ; while for a covalent environment,  $K<1$ . The lower the value of  $K$ , the greater is the covalency.

$$K_{\perp} = (g_{\perp} - 2.002) \Delta E_{xz} / 2\lambda_0 \quad (4)$$

$$K_{||} = (g_{||} - 2.002) \Delta E_{xy} / 8\lambda_0 \quad (5)$$

$$K^2 = (K_{2||} + 2K_{\perp}^2) / 3 \quad (6)$$

$K$  values (Table 2a), for the copper(II) complexes (2), (3) and (4) are indicating for a covalent bond character [34,51]. Kivelson and Neiman noted that, for ionic environment  $g_{||} \geq 2.3$  and for a covalent environment  $g_{||} < 2.3$  [52]. Theoretical work by Smith seems to confirm this view [50]. The  $g$ -values reported here (Table 2) show considerable covalent bond character [34]. Also, the in-plane  $\sigma$ -covalency parameter,  $\alpha^2(\text{Cu})$  was calculated by  $\alpha^2(\text{Cu}) = (A_{||}/0.036) + (g_{||} - 2.002) + 3/7(g - 2.002) + 0.04$  (7)

The calculated values (Table 2a) suggest a covalent bonding [34,51]. The in-plane and out of- plane  $\pi$ - bonding coefficients  $\beta_1^2$  and  $\beta^2$  respectively, are dependent upon the values of  $\Delta E_{xy}$  and  $\Delta E_{xz}$  in the following equations [45].

$$\alpha^2 \beta^2 = (g_{\perp} - 2.002) \Delta E_{xy} / 2\lambda_0 \quad (8)$$

$$\alpha^2 \beta_1^2 = (g_{||} - 2.002) \Delta E_{xz} / 8\lambda_0 \quad (9)$$

In this work, the complexes (2) and (3) show  $\beta_1^2$  values 1.65 and 0.85 indicating a moderate degree of covalency in the in-plane  $\pi$ -bonding [51,53].  $\beta^2$  value for complexes (2) and (3) show 2.29 and 1.68 indicating ionic character of the out-of-plane, while  $\beta^2$  value for complex (2) is 2.29 indicating a covalent bonding character out-of- plane  $\pi$ -bonding [51,53]. It is possible to calculate approximate orbital populations for orbitals [29] by

$$A_{||} = A_{iso} - 2B[1 \pm (7/4) \Delta g_{||}] \quad \Delta g_{||} = g_{||} - g_e \quad (10)$$

$$\alpha_{p,d}^2 = 2B / 2B^{\circ} \quad (11)$$

Where  $A^{\circ}$  and  $2B^{\circ}$  is the calculated dipolar coupling for unit occupancy of  $d$  orbital respectively. When the data are analyzed, the components of the Cu hyperfine coupling were considered with all the sign combinations [29]. The only physically meaningful results are found when  $A_{||}$  and  $A_{\perp}$  were negative. The resulting isotropic coupling constant was negative and the parallel component of the dipolar coupling  $2B$  are negative (-225 and -125 G). These results can only occur for an orbital involving the  $d_{x^2-y^2}$  atomic orbital on copper. The value for  $2B$  is quite normal for copper(II) complexes [54]. The  $|A_{iso}|$  value was relatively small. The  $2B$  value divided by

$2B^{\circ}$  (The calculated dipolar coupling for unit occupancy of  $d_{x^2-y^2}$  (-235.11 G), using equation (10) suggests all orbital population close to 53-96 %  $d$ -orbital spin density, clearly the orbital of the unpaired electron is  $d_{x^2-y^2}$ <sup>55</sup>. Complexes (4), (5) and (8) show an isotropic spectra with  $g_{iso} = 2.09$ -2.14 range.

**Table 2a:** ESR data for metal (II) complexes

Complex	$g_{  }$	$g_{\perp}$	$g_{iso}$	$A_{  }$ (G)	$A_{\perp}$ (G)	$A_{iso}$ (G)	$G$	$\Delta E_{xy}$ (cm <sup>-1</sup> )	$\Delta E_{xz}$ (cm <sup>-1</sup> )	$K_{  }^2$	$K_{\perp}^2$	$K^2$	$K$	$\alpha^2/\lambda_0$ (cm <sup>-1</sup> )	$\alpha^2$	$\beta^2$	$\beta_1^2$	$-2\beta$	$a^2/b^2$	
(2)	2.22	2.09	2.13	100	10	40	2.4	18518	20202	0.85	0.61	0.77	0.87	222	0.37	2.29	1.65	225	96	
(3)	2.2	2.08	2.12	120	15	50	2.5	17241	21505	1.01	0.51	0.84	0.91	183	0.601	1.68	0.85	125	53	
(4)	.	.	2.14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
(5)	.	.	2.09	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
(8)	.	.	2.11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

$$a_{iso} = (2g_{\perp} + g_{||}) / 3, \quad b_{iso} = (2A_{\perp} + A_{||}) / 3, \quad cG = (g_{||} - 2) / (g_{\perp} - 2) \quad (12), (13)$$

### 3.7 Thermal analyses (Differential Thermal Analysis (DTA) and Thermo Gravimetric Analysis (TGA))

Since the IR spectra indicate the presence of water molecules, thermal analyses (DTA and TGA) were carried out to certain their nature. The thermal curves in the temperature 27-600 $^{\circ}$ C range for complexes (12), (13), (14), (15) and (17) are thermally stable up to 45  $^{\circ}$ C. Broken of hydrogen bonding occurs as endothermic peak within the temperature 45-50  $^{\circ}$ C as shown in (Table 2b). Dehydration is characterized by endothermic peak at the temperature 80  $^{\circ}$ C, corresponding to the loss of hydrated water molecules. The elimination of coordinated water molecules occur in 115-150  $^{\circ}$ C range accompanied by endothermic peaks as in complexes (12), (13), (14), (15) and (17) [60,61]. The TG and DTA thermogram of Cu(II) complex (12) showed that, the complexes decomposed in five steps. The first occurred at 50 $^{\circ}$ C with no weight loss as endothermic peak, may be due to break of hydrogen bonding. The second step occurred at 80  $^{\circ}$ C with 9.59% weight loss (Calc. 9.70%) could be due to the elimination of three hydrated H<sub>2</sub>O another endothermic peak at 150  $^{\circ}$ C with 7.10% weight loss (Calc. 7.20%) are assigned to two coordinated water molecules. The TG curve displays another thermal decomposition at 260 $^{\circ}$ C with 12.40% weight loss (Calc. 12.70%), which could be due to the loss of acetate group. The complex shows an exothermic peak observed at 318 $^{\circ}$ C is due its melting point. Finally, exothermic peaks appear at 405, 450, 485, 565 and 580  $^{\circ}$ C corresponding to oxidative thermal decomposition which proceeds slowly with leaving CuO with 18.90 % weight loss (Calc. 18.98 %) <sup>62</sup>. The TG and DTA thermogram of Cu(II) Complex (14) shows endothermic peak at 75  $^{\circ}$ C, with 5.99 % weight loss (Calc. 6.12 %) due to loss of two hydrated water molecule and another endothermic peak at 115  $^{\circ}$ C with 5.50 % weight loss (Calc. 6.52%) are assigned to two coordinated water molecules. The endothermic peak observed at 245 and 265 $^{\circ}$ C with 18.50 % weight loss (Calc. 18.60 %), could be due

to the elimination of Sulphate group. Another exothermic peak observed at 325 with no weight loss may be due to its melting point. Finally, the complex shows exothermic peaks at 350,380,450,500 and 590°C with 18.89 % weight loss (Calc. 18.94 %) corresponding to oxidative thermal decomposition which proceeds slowly with final residue, assigned to CuO<sup>29</sup>. The TG and DTA thermogram of Zn(II) complex (17) shows endothermic peak at 50°C, due to break of hydrogen bonding. The endothermic peak observed at 85 °C with 5.99 % weight loss (Calc. 6.06 %), could be due to the elimination of the two hydrated H<sub>2</sub>O molecules. Another endothermic peak appeared at 150°C, with 3.20 % weight loss (Calc. 3.23 %), due to loss of one coordinated water molecule. The endothermic peak observed at 265 with 21.84 % weight loss (Calc. 21.86 %), could be due to the elimination of the two acetate groups. The complex displayed another exothermic peak at 420°C may be assigned to its melting point. Oxidative thermal decomposition occurs in the 450, 500, 550 and 590°C with exothermic peaks, leaving ZnO with 19.16% weight loss (Calc. 19.29 %) [62].

**Table 2b:** Thermal analyses for metal (II) complexes

NO.	Molecular formula	Temp. (°C)	DTA (peak)		TGA (Wt. loss %)		Assignments
			Endo	Exo	Calcd.	Found	
(2)	[(L) <sub>2</sub> Cu(CH <sub>3</sub> COO)(H <sub>2</sub> O)] <sub>2</sub> H <sub>2</sub> O	50	endo	-	-	-	Broken of H-bonding
		80	endo	-	9.70	9.59	Loss of (3H <sub>2</sub> O) hydrated water molecules
		130	endo	-	7.20	7.1	Loss of (2H <sub>2</sub> O) coordinated water molecules
		260	endo	-	12.70	12.4	Loss of acetate group
		318	-	exo	-	-	Melting point
		450,500,550,580,590	-	exo	18.98	18.90	Decomposition process with the formation of CuO
(3)	[(HL)Cu(CH <sub>3</sub> CH <sub>2</sub> H <sub>2</sub> O)] <sub>2</sub> H <sub>2</sub> O	45	endo	-	-	Broken of H-bonding	
		80	endo	-	6.80	6.78	Loss of (2H <sub>2</sub> O) hydrated water molecules
		135	endo	-	3.60	3.50	Loss of H <sub>2</sub> O coordinated water molecules
		245,265	endo	-	14.90	14.83	Loss of 2Cl <sup>-</sup> ions
		355	-	exo	-	-	Melting point
		405,450,485,565,580	-	exo	18.90	18.85	Decomposition process with the formation of CuO
(4)	[(HL) <sub>2</sub> Cu(SO <sub>4</sub> (H <sub>2</sub> O)) <sub>2</sub> ].2H <sub>2</sub> O	50	endo	-	-	Broken of H-bonding	
		75	endo	-	6.12	5.99	Loss of (2H <sub>2</sub> O) hydrated water molecules
		115	endo	-	6.52	5.50	Loss of (2H <sub>2</sub> O) coordinated water molecules
		245,265	endo	-	18.6	18.50	Loss of SO <sub>4</sub> group
		325	-	exo	-	-	Melting point
		350,380,450,500,590	-	exo	18.94	18.89	Decomposition process with the formation of CuO
(5)	[(HL) <sub>2</sub> Mn(CH <sub>3</sub> COO) <sub>2</sub> (H <sub>2</sub> O)] <sub>2</sub> H <sub>2</sub> O	45	endo	-	-	Broken of H-bonding	
		82	endo	-	6.17	6.09	Loss of (2H <sub>2</sub> O) hydrated water molecules
		130	endo	-	3.28	3.19	Loss of H <sub>2</sub> O Coordinated water molecules
		240,260	endo	-	22.29	22.21	Loss of the two acetate groups
		350	-	exo	-	-	Melting point
		400,450,490,560,590	-	exo	18.50	16.48	Decomposition process with the formation of MnO
(7)	[(HL) <sub>2</sub> Zn(CH <sub>3</sub> COO) <sub>2</sub> (H <sub>2</sub> O)] <sub>2</sub> H <sub>2</sub> O	50	endo	-	-	Broken of H-bonding	
		85	endo	-	6.06	5.99	Loss of (2H <sub>2</sub> O) hydrated water molecules
		130	endo	-	3.23	3.20	Loss of H <sub>2</sub> O coordinated water molecules
		265	endo	-	21.86	21.84	Loss of the two acetate groups
		420	-	exo	-	-	Melting point
		450,500,550,590	-	exo	19.29	19.16	Decomposition process with the formation of NiO

### 3.8 CHEMOTHERAPEUTIC STUDIES

The biological activity of the ligand (11) and its metal complexes (12), (13), (17), (18) and (19) were evaluated against HEPG-2 cell line. In this study, we try to know the chemotherapeutic activity of the tested complexes by comparing them with the standard drug (Vinblastine Sulfate). The treatment of the different complexes in DMSO showed similar effect in the tumoral cell line used as it was previously reported [63]. The solvent dimethyl sulphoxide (DMSO) shows no effect in cell growth. The ligand (11) shows a moderate inhibition effect at ranges of concentrations used, however, some

complexes showed better effect against HEPG-2 cell line. The obtained data indicate the surviving fraction ratio against HEPG-2 tumor cell line increasing with the decrease of the concentration in the range of the tested concentrations. Also, the Zn(II) complex (17) shows the highest potency of inhibition at 500 µg/ml against HEPG-2 cell line, compared with the standard drug [51]. Cytotoxicity results indicated that the tested complexes (13), (17) and (19) (IC<sub>50</sub> = 55.88–14.7 µg/ml) demonstrated potent cytotoxicity against HepG2 cancer cells Figures 6-8. Zn(II) complex (17) showed the highest cytotoxicity effect against HEPG-2 cell line with IC<sub>50</sub> value of 5.88 µg/ml, followed by Ag(I) complex (19) with IC<sub>50</sub> value 13.4 µg/ml. The cytotoxicity of the complexes (17) and (19) are more active than standard drugs used. This can be explained as metal ion binds to DNA. It seems that, changing the anion and the nature of the metal ion has effect on the biological behavior, due to alter binding ability of DNA binding, so testing of different complexes is very interesting from this point of view. Chemotherapeutic activity of the complexes may be attributed to the central metal atom which was explained by Tweedy's chelation theory [63,64]. Also, the positive charge of the metal increases the acidity of coordinated ligand that bears protons, leading to stronger hydrogen bonds which enhance the biological activity [65,66]. Moreover, Gaetke and Chow had reported that, metal has been suggested to facilitate oxidated tissue injury through a free-radical mediated pathway analogous to the Fenton reaction [66]. By applying the ESR-trapping technique, evidence for metal - mediated hydroxyl radical formation in vivo has been obtained [48]. ROS are produced through a Fenton-type reaction as follows:



Where L, organic ligand

Also, metal could act as a double-edged sword by inducing DNA damage and also by inhibiting their repair [67]. The OH radicals react with DNA sugars and bases and the most significant and well-characterized of the OH reactions is hydrogen atom abstraction from the C4 on the deoxyribose unit to yield sugar radicals with subsequent β-elimination (Scheme 2). By this mechanism strand break occurs as well as the release of the free bases. Another form of attack on the DNA bases is by solvated electrons, probably via a similar reaction to those discussed below for the direct effects of radiation on DNA [68]. In the ranges of concentrations used, the chemotherapeutic effect against HEPG-2 cell line are depicted in (Table 3), although, the complexes have the same anions, the variable activity of the complexes may be used to oxidation – reduction potentials. The cytotoxic effect of standard drugs and metal complexes in the ranges of concentrations used against human HEPG-2 cell line are shown in Figures 6-9.



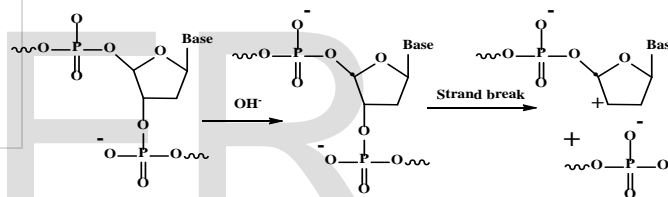
**Table 3:** Order of cytotoxic effect of the studied complexes against HEPG-2 cell line

concentration	Order of cytotoxic effect of studied complex (HEPG-2 cell line)
500 µg/ml	(7)>(1)>(9) >std.>(3)>(2).>(8)
125 µg/ml	(7)>(1)>(9) >std.>(3)>(2).>(8)
31.25 µg/ml	std.>(7)>(1)>(9) >(3)>(2) >(8)
7.8 µg/ml	std.>(7)>(1)>(9) >(3)>(2) >(8)
2.0 µg/ml	std.>(7)>(1)>(9) >(3)>(8),(2)
1.0 µg/ml	std.>(7)>(1)>(9) >(3)>(8),(2)

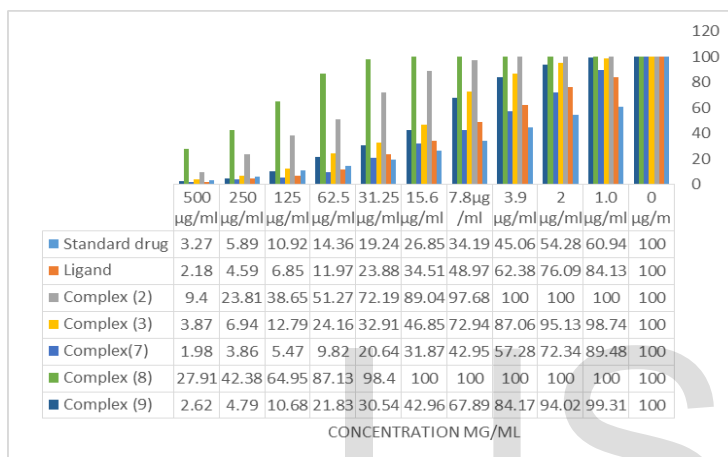
Moreover, Gaetke and Chow had reported that, metal has been suggested to facilitate oxidated tissue injury through a free-radical mediated pathway analogous to the Fenton reaction [66]. By applying the ESR-trapping technique, evidence for metal - mediated hydroxyl radical formation in vivo has been obtained [48]. ROS are produced through a Fenton-type reaction as follows:



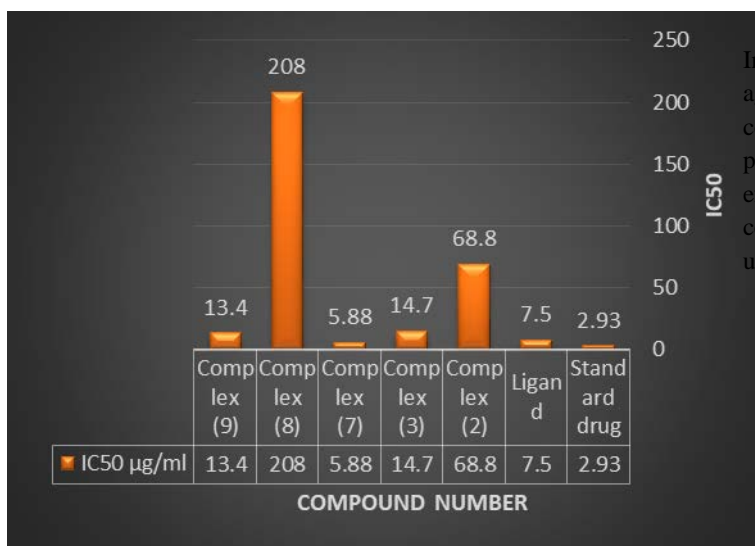
Where L, organic ligand. Also, metal could act as a double-edged sword by inducing DNA damage and also by inhibiting their repair [67]. The OH radicals react with DNA sugars and bases and the most significant and well-characterized of the OH reactions is hydrogen atom abstraction from the C4 on the deoxyribose unit to yield sugar radicals with subsequent β-elimination (Scheme 2). By this mechanism strand break occurs as well as the release of the free bases. Another form of attack on the DNA bases is by solvated electrons, probably via a similar reaction to those discussed below for the direct effects of radiation on DNA [68].



**Scheme 2:** Suggested mechanism for OH radicals attack on DNA sugars and bases

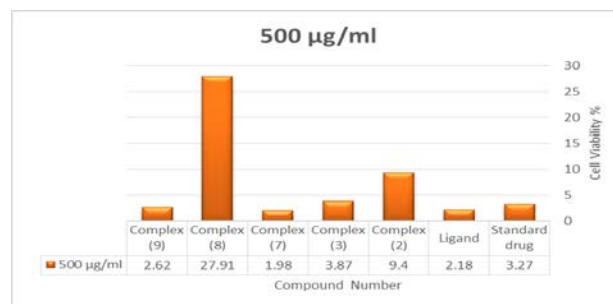


**Fig. 6:** Evaluation of cytotoxicity of metal complexes Against human hepatic HEPG-2 Cell Line

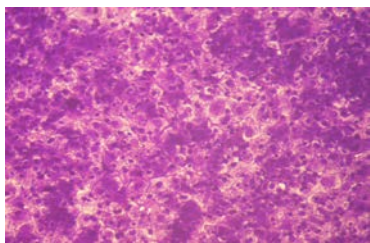


**Fig. 7:** IC<sub>50</sub> values of the ligand, HL (1) and its metal complexes against human hepatic HEPG-2 cell lines.

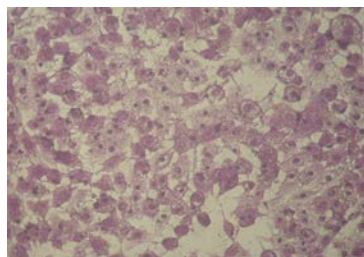
In the ranges of concentrations used, the chemotherapeutic effect against HEPG-2 cell line are depicted in (Table 3), although, the complexes have the same anions, the variable activity of the complexes may be used to oxidation – reduction potentials. The cytotoxic effect of standard drugs and metal complexes in the ranges of concentrations used against human HEPG-2 cell line are shown in Figures 6-9.



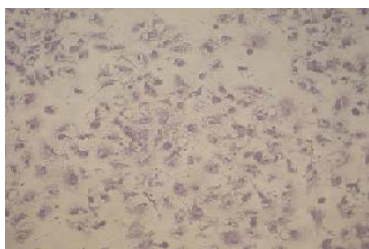
**Fig. 8:** Evaluation of cytotoxicity of metal complexes Against human hepatic HEPG-2 Cell Line at 500 µg/ml



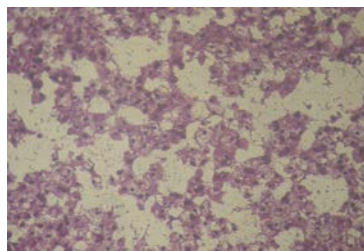
(A) Non treated against human hepatic HEPG-2 Cell Line



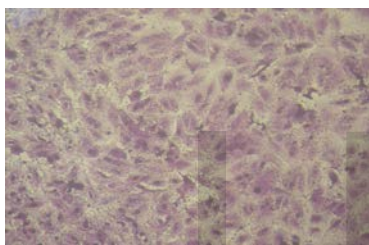
(F) HepG-2 cells treated with Ligand (1) at 250 ug/ml



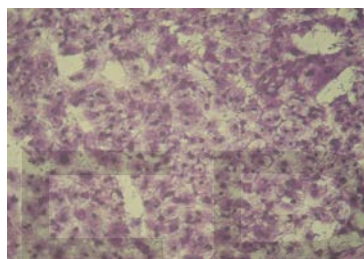
(B) HepG-2 cells treated with standard drug at 500 ug/ml



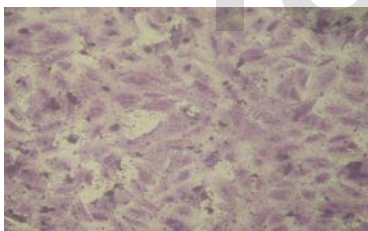
(G) HepG-2 cells treated with Complex (2) at 3.9 ug/ml



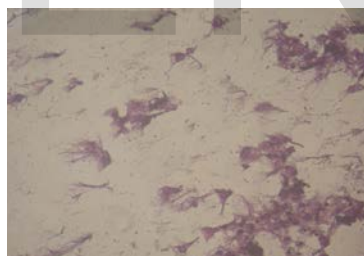
(C) HepG cells treated with Ligand (1) at 3.9 ug/ml



(H) HepG-2 cells treated with Complex (2) at 15.6 ug/ml



(D) HepG-2 cells treated with Ligand (1) at 15.6 ug/ml



(I) HepG-2 cells treated with Complex (2) at 62.5 ug/ml



(E) HepG-2 cells treated with Ligand (1) at 62.5 ug/ml



(J) HepG-2 cells treated with Complex (2) at 250 ug/ml

**Fig.9:** Histogram of non-treated and treated HEPG-2 Cell Line with standard, Ligand and complex (2)

### 3.9 ANTIMICROBIAL ACTIVITY

In vitro biological screening tests of the ligand and metal complexes (2), (3), (7) and (9) carried out as antibacterial and antifungal activity and presented in figure 19. The antibacterial activity was tested against two bacterial strains; Gram-positive *Staphylococcus aureus* and *Bacillus subtilis* as well as Gram-negative *Escherichia coli* and *Proteus vulgaris* strains [69]. The results compared with standard drug (Ampicillin (Gram positive) and Gentamicin (Gram negative)). The data indicated that, complexes were active against bacteria. Complexes of Cu(II) (2) and (3) and Zn(II) complex (8) and Ag(II) Complex (9) show antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *E.coli* and *Proteus vulgaris*<sup>80</sup>. The results showed that, the order of cytotoxic effect against Gram positive and Gram negative strains for *Staphylococcus aureus* is Standard > (2)>(3)>(9)>(7)>Ligand (1), for *Bacillus subtilis* the order is Standard >Ligand (1)>(2)>(3)>(9)>(7). Also for *Escherichia coli* the order is Standard > (2)>(3),(9)>(7)>Ligand (1) and also for *Proteus vulgaris* the order is (2)>Standard >Ligand (1)>(3)>(9)>(7). Cu(II) complexes showed wide range of bactericidal activities against the gram positive and gram negative bacteria. The complexes were also subjected to antifungal activity against *Aspergillus fumigatus* and *Candida albicans*. The investigation shows that, all tested complexes in general have cytotoxic activity against *Aspergillus fumigatus* and *Candida albicans*. Analysis of growth pattern of *Aspergillus fumigatus* in the presence of complexes reveals that, the complexes have potential to inhibit the growth of fungus tested. Further, Cu(II) complex (2) is more active than the free ligand, which indicates that, metalation increases antimicrobial activity. Cu(II) complex (2) and Ag(II) complex (9) are more active against *Aspergillus fumigatus* and *Candida albicans* whereas Cu(II) complex (4) and Zn(II) (7) are moderately active against *Candida albicans*. Also Zn(II) complex (7) is not active against *Aspergillus fumigatus*. The results showed that, the order of cytotoxic effect for *Aspergillus fumigatus* is (2)>Ligand (1) > (9)>standard>(9)>(3)>(7) and for *Candida albicans* is (2)>Ligand (1)>standard>(3)>(9)>(3)>(7).The complexes of (Z)-3-((2-((2-hydroxybenzylidene)amino)phenyl)amino)-3,4-dihydroquinoxalin-2(1H)-one are more active for microorganisms and this is also indicates that, nitrogen is a more effective antimicrobial agent. The increase in the activity of complexes as compared to the parent ligand may be due to the complex formation in which the ligand is coordinated to the central metal ion through the azomethine

nitrogen leading to an increased biological action. The zone of inhibition was measured with respect to control.

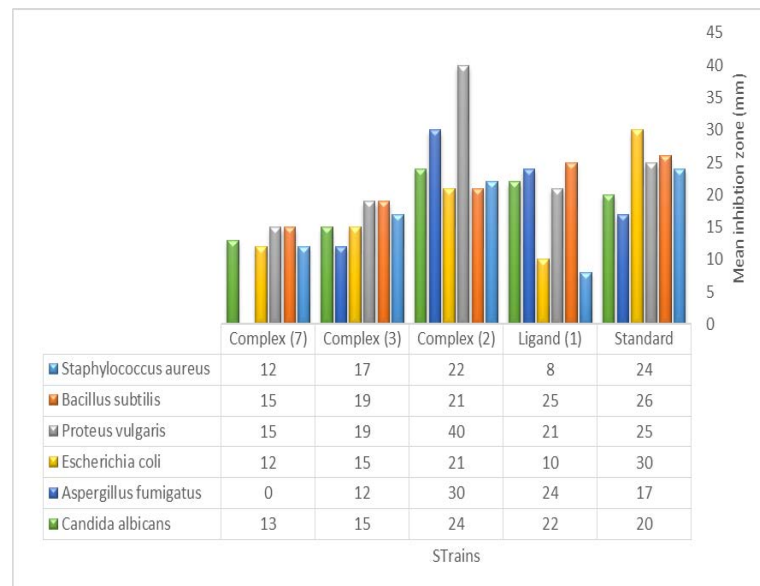


Fig. 19: Mean inhibition zone of the ligand (1) and metal complexes (2), (3) and (7) against *Staphylococcus aureus*, *Bacillus subtilis*, *Proteus vulgaris*, *Escherichia coli*, *Aspergillus fumigatus* and *Candida albicans*

### 4 CONCLUSION

Novel Schiff base ligand, Z)-3-((2-((2-hydroxybenzylidene)amino)phenyl)amino)-3-dihydroquinoxalin-2(1H)-one and its Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Ag(I) complexes were synthesized. The analytical and physicochemical data confirmed the composition and structure of the newly obtained compounds. The ESR spectra of solid Cu(II) complexes (2-4) show an anisotropic or isotropic type indicating a  $d(x^2-y^2)$  ground state with a significant covalent bond character. However, Mn(II) complex (5) and Co(II) Complex (8) show an isotropic type indicating an octahedral geometry. Cytotoxic evolution  $IC_{50}$  of the ligand and its complexes have been carried out. Zn(II) complex (7) and Ag(I) complex (9) show enhanced activity in comparison to the parent ligand or standard drug. Cu(II) complexes (2) and (3) have been done. Copper is enriched in various human cancer tissues and is a co-factor essential for tumor angiogenesis processes. However, the use of copper binding ligand to target tumor, copper could provide a novel strategy for cancer selective treatment. Also, antimicrobial studies of the ligand and some of its complexes are described. These compounds are promising candidates as anticancer agents and antimicrobial because of their high cytotoxic activities.

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