Modulation of cancer therapy using nano-organometallic compounds: preparation, spectroscopic characterization and cytotoxic evaluation

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Abstract

New series of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) organometallic complexes with hydroxyl benzylidene malonohydrazide ligand have 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. The establishment of structure activity relationships and elucidation of the specification of complexes under conditions relevant to drug testing and formulation are crucial for the further development of promising medicinal applications of organometallic complexes. Specific examples involving the design of metal complexes as anticancer agents are discussed. These complexes have been synthesized and characterized by (1H-NMR, mass, IR, UV-VIS and ESR) spectroscopy, as well as magnetic moments, conductance, elemental and thermal analyses. Molar conductance in DMF solution indicates that, the complexes are non-electrolytes. The ESR spectra of solid Cu(II) complexes (7) and (8) showed isotropic and anisotropic types indicating an octahedral geometry with covalent bond character. However, Co(II) complexes (3) and (4) showed anisotropic type where, g┴ > g|| >2.0023, indicating compressed tetragonal distortion around Co(II) ion. Cytotoxic evolution of the ligand and its complexes have been carried out. Complexes showed enhanced activity in comparison to the parent ligand or standard drug applied.

1. Introduction

Schiff-bases have played a key role in the development of coordination chemistry. Schiff- base compounds containing an imine group are usually formed by the condensation of a primary amines with an active carbonyl or aldehyde group. These compounds are considered as a very important class of organic compounds, which have wide applications in many biological spects. Various Schiff–bases complexes were reported to possess geno-toxicity, antibacterial and antifungal activities. The increasing interest in transition metal complexes containing Schiff-base ligand is derived from their well-established role in biological systems as well as their catalytic and pharmaceutical application. Metal complexes provide a highly versatile platform for drug design. Besides variation in the metal and its oxidation state, that allow the fine-tuning of their chemical reactivity in terms of both kinetics and thermodynamics. Not only the metal but also the ligands can play important roles in biological activity, ranging from outer-sphere recognition of the target site to the activity of any released ligands and ligand centered redox processes. Due to a growing interest in the in the development of metallo-therapeutic drugs and metal-based agents, we reported herein...
synthesis and characterization of new metallo-
therapeutic candidates derived from
the novel ligand \((N^1E,N^3E)-N^1,N^3\) bis
(2-hydroxybenzylidene) malonohydrazide. The
 cytotoxic activity of synthesized compounds has been
also investigated.

2. Experimental

Instrumentation and measurement:

The ligand and its complexes were synthetic grade and
used without further purification. 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\(_4\)O\(_{10}\).
The IR spectra were measured as KBr pellets using a
Perkin-Elmer 683 spectrophotometer (4000-400 cm\(^{-1}\)). Electronic spectra (qualitative) were recorded on a
Perkin-Elmer 550 spectrophotometer. The
conductance(10\(^{-5}\)M) of the complexes in DMF were
measured at 25 °C with a Bibby conduct meter type
MCl. \(^1\)H-NMR spectra of the ligand and its Cd(II)
complex 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
tetraethyl arsoniobalt(II) as the magnetic
susceptibility standard. Diamagnetic corrections
were estimated from Pascal's constant\(^{13}\). The magnetic
moments were calculated from the equation: 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.

Transmission electron microscope (TEM): TEM
samples for the colloidal suspensions of the complexes
in dis. water were prepared by dropping the colloids
onto carbon-coated TEM grids (Carbon coated Cu
grids, Ted Pella, Redding, CA, USA) and allowing the
liquid carrier to evaporate in air then assaying by a
JEOL 1230 transmission electron microscope (120
ev).

BIOLOGICAL ACTIVITY

Cytotoxic activity: Evaluation of the cytotoxic
activity of the ligand and some of 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 (10\(^4\)cells/well) for 24 hrs.
Before treatment with the complexes to allow
attachment of cell to the wall of the plate. Different
centrations 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 using
5% CO\(_2\). 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.

Scheme (1) Preparation of the ligand
Synthesis of metal complexes (2)-(13): The metal complexes 2-13 were prepared by refluxing with stirring a suitable amount of a hot ethanolic solution of the following metal salts: Mn(OAc)₂·4H₂O, Co(OAc)₃, 4H₂O, CoSO₄·7H₂O, CoCl₂·6H₂O, Ni(OAc)₂·4H₂O, Cu(OAc)₂(H₂O)₂, CuSO₄·5H₂O, CuCl₂·2H₂O, Cu(NO₃)₂·2H₂O, CuCO₃, Zn(OAc)₂·2H₂O, Cd(OAc)₂·2H₂O and then added to hot ethanolic solution of the ligand with molar ratio (2 metal: 1 ligand). The refluxing times varied from 2 to 4 hours according to the depending to nature of metal ion. The precipitates which formed were filtered off, washed with ethanol then by diethyl ether and dried in vacuum desiccators over P₂O₅. Structure representation and analytical data for the prepared complexes are shown in figures 1 and 2 table 1.

3. Results and Discussion

All the metal complexes are stable at room temperature, no hydroscopic, insoluble in water, partially soluble in MeOH, EtOH, CHCl₃ and (CH₃)₂CO and completely soluble in DMF and DMSO. The analytical and physical data, spectral data are compatible with the proposed structures, figure 1 and 2. The molar conductance of the complexes in 10⁻³ M DMF at 25 °C are in the 4.77-1.09 Ωm⁻¹cm²mol⁻¹ range, indicating a non-electrolytic nature. These low values commensurate the absence of any counter ions in their structure. Many attempts were made to grow a single crystal but unfortunately, they were failed. Reaction of the ligand (1) with metal salts using (1L: 2M) molar ratio in ethanol gives complexes (2)-(13).

Table 1: Analytical and Physical Data of the Ligand [ H2L ] (1) and its Metal Complexes.

<table>
<thead>
<tr>
<th>No.</th>
<th>Ligand/Complexes</th>
<th>Color</th>
<th>FW (g/mol)</th>
<th>M.P (ºC)</th>
<th>Yield (%)</th>
<th>Anal. /Found (Calc.) (%)</th>
<th>Molar conductance (Ω⁻¹ cm² mol⁻¹)</th>
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<tr>
<td>(1)</td>
<td>[H₃L] C₁₇H₁₆N₄O₄</td>
<td>Reddish brown</td>
<td>340.33</td>
<td>&gt;300</td>
<td>75</td>
<td>59.99(59.52)</td>
<td>4.74(4.61)</td>
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<td>(2)</td>
<td>[(H₃L)Mn₂][OAc]₃ (H₂O)₂·2H₂O C₂₀H₂₀Mn₃N₄O₁₆</td>
<td>Pale brown</td>
<td>722.42</td>
<td>&gt;300</td>
<td>60</td>
<td>41.56(41.1)</td>
<td>4.46(4.23)</td>
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<td>(3)</td>
<td>[(H₃L)Co][OAc]₄ (H₂O)₂·H₂O C₂₀H₂₀Co₄N₄O₄S₂</td>
<td>Gray</td>
<td>730.41</td>
<td>&gt;300</td>
<td>65</td>
<td>41.11(40.95)</td>
<td>4.42(4.0)</td>
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<tr>
<td>(4)</td>
<td>[(H₃L)Co][SO₄]·3(H₂O)₄·H₂O C₂₀H₂₀Co₄N₄O₄S₂</td>
<td>Yellowish brown</td>
<td>722.39</td>
<td>&gt;300</td>
<td>70</td>
<td>28.26(27.92)</td>
<td>3.35(3.01)</td>
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<td>(5)</td>
<td>[(H₃L)Co][Cl]·3(H₂O)₄·H₂O C₂₀H₂₀Co₄N₄O₄S₂</td>
<td>Dark brown</td>
<td>636.04</td>
<td>&gt;300</td>
<td>73</td>
<td>32.10(31.87)</td>
<td>3.17(3.02)</td>
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<td>(6)</td>
<td>[(H₃L)Ni][OAc]₃ (H₂O)₂·3H₂O C₂₀H₂₀Ni₄N₄O₄</td>
<td>Brown</td>
<td>729.93</td>
<td>&gt;300</td>
<td>80</td>
<td>41.14(40.89)</td>
<td>4.31(4.42)</td>
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<td>(7)</td>
<td>[(H₃L)Cu][OAc]₄ (H₂O)₂·H₂O C₂₀H₂₀Cu₄N₄O₄</td>
<td>Rose</td>
<td>737.62</td>
<td>&gt;300</td>
<td>70</td>
<td>40.71(40.32)</td>
<td>4.42(4.31)</td>
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<td>(8)</td>
<td>[(H₃L)Cu][SO₄]·3(H₂O)₄·H₂O C₂₀H₂₀Cu₄N₄O₄</td>
<td>Gray</td>
<td>731.61</td>
<td>&gt;300</td>
<td>85</td>
<td>27.91(27.59)</td>
<td>3.31(3.11)</td>
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<td>(9)</td>
<td>[(H₃L)Cu][Cl]·3(H₂O)₄·2H₂O C₂₀H₂₀Cu₄N₄O₄S₂</td>
<td>Greenish yellow</td>
<td>645.27</td>
<td>&gt;300</td>
<td>69</td>
<td>31.64(31.25)</td>
<td>3.12(3.02)</td>
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<td>(10)</td>
<td>[(H₃L)Cu][NO₃]·3(H₂O)₄·H₂O C₂₀H₂₀Cu₄N₄O₄</td>
<td>Greenish brown</td>
<td>751.48</td>
<td>&gt;300</td>
<td>68</td>
<td>27.17(26.95)</td>
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<td>(11)</td>
<td>[(H₃L)Cu][CO₃]·3(H₂O)₄·H₂O C₂₀H₂₀Cu₄N₄O₄S₂</td>
<td>Green</td>
<td>743.52</td>
<td>&gt;300</td>
<td>75</td>
<td>33.92(33.81)</td>
<td>2.71(2.56)</td>
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<tr>
<td>(12)</td>
<td>[(H₃L)Zn][OAc]·3(H₂O)₄·H₂O C₂₀H₂₀Cu₄N₄O₄S₂</td>
<td>Dark green</td>
<td>743.38</td>
<td>&gt;300</td>
<td>69</td>
<td>40.40(40.20)</td>
<td>4.34(4.11)</td>
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<td>(13)</td>
<td>[(H₃L)Cd]OAc]₃·3(H₂O)₄·2H₂O C₂₀H₂₀Cd₃N₄O₄</td>
<td>Pale brown</td>
<td>837.36</td>
<td>&gt;300</td>
<td>75</td>
<td>35.86(35.61)</td>
<td>3.85(3.55)</td>
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</table>

* Δm (Ω⁻¹ cm² mol⁻¹)
**1H-NMR spectra of the ligand (1) and Zn(II) complex (12)**: The 1H-NMR spectra of ligand and Zn(II) complex (12) in deuterated DMSO showed peaks consistent with the proposed structure (Scheme 1 and Figure 2). The 1H-NMR spectrum of the ligand showed chemical shift observed as singlet at 9.96 ppm (s, 2H, OH) which is assigned to proton of aromatic hydroxyl group. The chemical shifts which appeared at 8.8-8.9 ppm range was attributed to the azomethine protons (12-H-C=N). However, the chemical shifts appeared as a singlet at 6.95 ppm is attributed to the proton of NH attached to carbonyl group. A set of signals appeared as multiples in the 7.09-7.3 ppm range, corresponding to protons of aromatic ring. By comparison the 1H NMR of the ligand and the spectrum of the Zn(II) complex (12),
presence of the signal characteristic to the OH group appeared at 10.3 ppm 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 attached to carbonyl group 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→Zn)16,17.

**Mass spectra:** The mass spectra of (1) and its, Cu(II) complex, (9) confirmed their proposed formulation. The spectrum of (1) reveals the molecular ion peak (m/z) at 340 amu consistent with the molecular weight of the ligand. Furthermore, the fragments observed at (m/z) = 59, 72, 90, 140, 170 and 338 amu correspond to C6H11, C6H12, C6H14O, C6H14NO, C6H14N2O2 and C7H14N4O4 moieties respectively. Complex (9) showed fragments (m/z) at 60, 72, 84, 130, 240 and 379 amu due to C6H12, C6H12, C6H12, C7H14NO, C13H19ClNO and C16H12ClCuN2O3 moieties respectively.

**IR Spectra:** 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)-(13) as shown in table 2. The ligand showed bands in the 3660-3345 and 3340-2680 cm⁻¹ ranges, commensurate the presence of two types of intra- and intermolecular hydrogen bonds of OH and NH groups with imine group26. Thus, the higher frequency band is associated with a weaker hydrogen bond. The medium band at 3190 cm⁻¹ is assigned to ν(NH) groups18,19. The ν(NH) group in the complexes appeared nearly at the same region of the free ligand indicating that, the NH group is not involved in the coordination to the metal ion20. However, the characteristic bands of imines, ν(C=N), ν(C=O) and ν(C-OH) were observed at 1662, 1735 cm⁻¹ respectively. Strong band appeared at 1330-1240 cm⁻¹ range is attributed to the ν(C-OH) vibration. The bands appeared at 1572-1458 and 780-750 cm⁻¹ range, are assigned to ν(Ar) vibration20,21. The ν(N-N) group appears at 1043 cm⁻¹. By comparing the IR spectra of the complexes (2)-(13) with that of the free ligand. It was found that, the position of the ν(C=N) bands of imines is shifted by 8-42 cm⁻¹ range towards lower wave number in the complexes indicating coordination through nitrogen of azomethine group (CH=N)20,23. This is also confirmed by the appearance of new bands in the 586-516 cm⁻¹ range, this has been assigned to the υ(M-N)23. Complexes (2)-(13) showed υ(C-OH) in the 1385-1149 range, indicating coordination to the metal ion24. The aromatic ring to the metal ion appeared in the 1575-1455 cm⁻¹ and 870-740 cm⁻¹ ranges24. The IR spectra of the metal complexes (2)-(19) showed bands in the 3650-3610 cm⁻¹, 3360-3230 cm⁻¹, 3350-3210 cm⁻¹ and 2875-2550 cm⁻¹ 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, bidentate or bridging bidentate manner. The υas(CO2) and υs(CO2) of the free acetate ion are ca. 1560 and 1416 cm⁻¹ respectively. In unidentate acetate complexes υ(C=O) is higher than υs(CO2) and υ(C-O) is lower than υas(CO2). As a result the separation between the two υ(CO) is much larger in unidentate than in free ion but in bidentate the separation is lower than in the free ion while in bridging bidentate the two υ(CO) is closer to the free ion25. In the case of acetate complexes (2), (3), (6), (7), (12) and (13) showed bands in the 1480-1430 and 1360-1326 cm⁻¹ 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 υasym(COO) and υsym(COO). The separation value (Δ) between υasym(COO) and υsym(COO) in this complex was in the 120-104 cm⁻¹ range suggesting the coordination of acetate group in these complexes as a monodentate fashion18,23. The sulphato complex (4) showed bands at 1285, 1180, 1041 and 740 cm⁻¹ and complex (8) showed bands at 1252, 1167, 1082 and 750 cm⁻¹ which assigned to monodentatesulphate group26. Complexes (2)-(13) showed bands in the 586-516 cm⁻¹ is assigned to υ(M-N)23. Complexes (2)-(13) showed bands in the 678-612 cm⁻¹ are due to υ(M-O)25.

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Table 2.- IR Frequencies of the Bands (cm⁻¹) of the Ligand [H₂L]₁ (1) and its Metal complexes:

<table>
<thead>
<tr>
<th>No.</th>
<th>ν(H₂O)</th>
<th>ν(OH)</th>
<th>ν(H-bonding)</th>
<th>ν(C=N)</th>
<th>ν(C=O)</th>
<th>ν(NH)</th>
<th>ν(COH)</th>
<th>ν(Ar)</th>
<th>ν(Ο/Ο /ΝΟ)</th>
<th>ν(M-O)</th>
<th>ν(M-N)</th>
<th>ν(M-Cl)</th>
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<td>(1)</td>
<td>-</td>
<td>3470-3374</td>
<td>3660-3345,3335-3280</td>
<td>1662</td>
<td>1735</td>
<td>3190</td>
<td>1330-1340</td>
<td>1572.783</td>
<td>1485.750</td>
<td>-</td>
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<td>(2)</td>
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<td>1620</td>
<td>1795</td>
<td>3180</td>
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<td>781.749</td>
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<td>678</td>
<td>562*</td>
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<td>1658</td>
<td>1725</td>
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<td>577</td>
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<td>3340-3270-3095</td>
<td>3453</td>
<td>3630-3310,3280-2690</td>
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</table>

**Magnetic moments**: The magnetic moments of the metal complexes (2)–(13) at room temperatures are shown in (Table 3). Copper(II) complexes (7) and (11) show values between 1.67 – 1.70 B.M. corresponding to one unpaired electron in an octahedral structure. Cobalt(II) complexes (3), (4) and (5) showed values 4.38,4.85 and 4.42 B.M., indicating high spin octahedral cobalt(II) complexes. Nickel(II) complex (6) showed 3.02 B.M., indicating octahedral Ni(II) complex. Manganese (II) complex (2) gave 6.3 B.M., indicating high spin Mn(II) octahedral structure. Zn(II) complex (12) and Cd(II) complex (13) showed diamagnetic property.

**Electronic spectra and magnetic moments**: The electronic spectral data for the ligand (1) and its metal complexes in DMF solution are summarized in (Table 3). Ligand (1) in DMF solution showed two bands at 320 nm (ε = 7.72 x 10⁻³ mol⁻¹ cm⁻¹) and 295 nm (ε = 7.12 x 10⁻³ mol⁻¹ cm⁻¹) which may be assigned to n→π* and π→π* transitions of the imine and aromatic ring respectively. Cobalt(II) complexes (3) , (4) and (5) showed bands in the...
The ESR spectral data for complexes (2), (3), (4), (5), (7) and (8), (9), (10), (11) are presented in (Table 4). The spectra of Co(II) complexes (3), (4) and (5) showed anisotropic and isotropic type where, \( g_\perp > g_\parallel > 2.0023 \), indicating compressed tetragonal distortion around Co(II) ion. However, complex (5) showed isotropic type with \( g_{iso} = 2.04 \). The spectra of copper(II) complexes (8) and (11) are characteristic of species \( d^9 \) configuration having axial type of a \( g_{(x2,y2)} \) ground state which is the most common for copper(II) complexes\(^{34,35} \). The complexes showed \( g_\parallel > g_\perp > 2.0023 \), indicating distorted

Electron spin resonance (ESR)

The ESR spectral data for complexes (2), (3), (4), (5), (7) and (8), (9), (10), (11) are presented in (Table 4). The spectra of Co(II) complexes (3), (4) and (5) showed anisotropic and isotropic type where, \( g_\perp > g_\parallel > 2.0023 \), indicating compressed tetragonal distortion around Co(II) ion. However, complex (5) showed isotropic type with \( g_{iso} = 2.04 \). The spectra of copper(II) complexes (8) and (11) are characteristic of species \( d^9 \) configuration having axial type of a \( g_{(x2,y2)} \) ground state which is the most common for copper(II) complexes\(^{34,35} \). The complexes showed \( g_\parallel > g_\perp > 2.0023 \), indicating distorted
octahedral geometry around copper(II) ion. The g-values are related by the expression \( G = (g_{||} - 2)/ (g_{\perp} - 2) \), where \( G \) exchange coupling interaction parameter \( (G) \). If \( G > 4.0 \), a significant exchange coupling is present, whereas if \( G \) value > 4.0, local tetragonal axes are aligned parallel or only slightly misaligned. Complexes showed values indicating spin-exchange interactions take place between copper(II) ions. This phenomena is further confirmed by the magnetic moments values (Table 3). On the other hand, complex (7) showed isotropic type with \( g_{iso} = 2.15 \) indicating tetragonal axes are present in this complex. The \( g_{\parallel}/A_{\parallel} \) value is also considered as a diagnostic term for stereochemistry, the \( g_{\parallel}/A_{\parallel} \) values in the (105-135 cm\(^{-1}\)) range are expected for copper complexes within perfectly square planar geometry and for tetragonal distorted octahedral complexes are 150-250 cm\(^{-1}\). The \( g_{\parallel}/A_{\parallel} \) values for the copper complexes are 150.2, 181.1, 220 and 222 cm\(^{-1}\) which lie just within the range expected for the tetragonal distorted octahedral copper(II) complexes (Table 4). The g-value of the copper(II) complexes with a \( \beta \)B\( g \) ground state \((g_{\parallel} > g_{\perp})\) may be expressed by 

\[
g_{\parallel} = 2.002 - (8K_{1}^{2} \lambda_{1} / \Delta E_{xy}) \tag{1}
g_{\perp} = 2.002 - (2K_{2}^{2} \lambda_{1} / \Delta E_{xz}) \tag{2}
\]

Where \( K_{1} \) and \( K_{2} \) are the parallel and perpendicular components respectively of the orbital reduction factor \( (K) \), \( \lambda_{1} \) is the spin-orbit coupling constant for the free copper, \( \Delta E_{xy} \) and \( \Delta E_{xz} \) are the electron transition energies of \( \beta B_{1g} \rightarrow \beta B_{2g} \) and \( \beta B_{1g} \rightarrow \beta E_{g} \). From the above relations, the orbital reduction factors \( (K_{1}, K_{2}, K) \), which are measure terms for covalency, can be calculated. For an ionic environment, \( K=1 \); while for a covalent environment, \( K<1 \). The lower the value of \( K \), the greater the covalency.

\[
K_{1}^{2} = (g_{\parallel} - 2.002) \Delta E_{xz} / 2\lambda_{1o} \tag{3}
K_{2}^{2} = (g_{\perp} - 2.002) \Delta E_{xy} / 3\lambda_{1o} \tag{4}
K_{2}^{2} = (K_{3}^{2} + 2K_{2}^{2}) / 3 \tag{5}
\]

K values (Table 4), for the copper(II) complexes (8) - (11) are indicating for a covalent bond character. Kivelson and Neiman noted that, for ionic environment \( g_{iso} = 2.3 \) and for a covalent environment \( g_{iso} < 2.3 \). Theoretical work by Smith seems to confirmed this view. The g-values reported here (Table 4) show considerable covalent bond character. Also, the in-plane \( \sigma \)-covalency parameter, \( \alpha^{2}(\text{Cu}) \) was calculated by 

\[
\alpha^{2}(\text{Cu}) = (A_{\perp}/0.036) + (g_{\parallel} - 2.002)^{2} / (7(g_{\perp} - 2.002) + 0.04 \tag{6}
\]

The calculated values (Table 4) suggested a covalent bonding. The in-plane and out-of-plane \( \pi \)-bonding coefficients \( \beta^{2} \) and \( \beta^{2} \) respectively, are dependent upon the values of \( \Delta E_{xy} \) and \( \Delta E_{xz} \) in the following equations:

\[
\alpha^{2} \beta^{2} = (g_{\parallel} - 2.002) \Delta E_{xy} / 2\lambda_{1o} \tag{7}
\alpha^{2} \beta^{2} = (g_{\perp} - 2.002) \Delta E_{xz} / 3\lambda_{1o} \tag{8}
\]

In this work, complexes showed \( \beta^{2} \) values 0.9, 0.8 and 0.93 indicating a moderate degree of covalency in the in-plane \( \pi \)-bonding. \( \beta^{2} \) value for complexes showed 1.79, 1.08, 1.6 and 1.13 indicating ionic character of the out-of-plane. It is possible to calculate approximate orbital populations for d orbitals by 

\[
A_{\parallel} = A_{iso} - 2B [1 \pm (7/4) \Delta g] \quad \Delta g = g_{\parallel} - g_{\perp} \tag{9}
\alpha_{d,p} = 2B / 2B^{\circ} \tag{10}
\]

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 \( ^{45}\text{Cu} \) hyperfine coupling were considered with all the sign combinations. The only physically meaningful results are found when \( A_{\perp} \) and \( A_{\parallel} \) were negative. The resulting isotropic coupling constant was negative and the parallel component of the dipolar coupling 2B are negative (-125, -233, 212 and \(-178.1 \) 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. 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 53-99.1 \% d-orbital spin density, clearly the orbital of the unpaired electron is \( d_{x^{2}-y^{2}} \). Manganese(II) complex (2) showed isotropic type with \( g_{iso} = 2.02 \), indicating octahedral structure.

**Thermal analyses (DTA and TGA):** Since the IR spectra indicated 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 °C range for complexes (6), (9), (10), and (12) are thermally stable up to 45 °C. Broken of hydrogen bonding occurs as endothermic peak within the temperature 45-50 °C as shown in (Table 5). Dehydration is characterized by endothermic peaks within the

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Egypt. J. Chem. **64**, No. 7 (2021)
temperature 55-65°C range, corresponding to the loss of hydrated water molecules. The elimination of coordinated water molecules occurred in 120-130°C range accompanied by endothermic peaks\(^{49,50}\).

The TGA and DTA thermogram of Ni(II) complex (6) showed that, the complex decomposed in six steps. The first occurred at 45°C with no weight loss as endothermic peak, may be due to break of hydrogen bondings. The second step occurred at 55°C with 6.72% weight loss (Calc. 6.52%) as endothermic peak which could be due to the elimination of three hydrated water molecules.

The decomposition step which occurred at 120°C with 7.01% weight loss (Calc. 6.9%) could be due to the elimination of two coordinated H\(_2\)O. The TGA curve displayed another thermal decomposition at 230°C with 34.2% weight loss (Calc. 34.0%), which could be due to the loss of four coordinated acetate groups. The complex showed an exothermic peak observed at 350°C was due to its melting point. Finally, exothermic peaks appeared at 405, 450, 485, 565 and 580°C corresponding to oxidative thermal decomposition which proceeded slowly with leaving 2NiO with 32.8% weight loss (Calc. 32.6%)\(^{51}\).

Oxidative thermal decomposition occurs in 450, 500, 550, 570 and 590°C with 34.8% weight loss (Calc. 34.5%) exothermic peaks, leaving 2ZnO\(^{51}\).

**Transmission electron microscope characterization (TEM):** The average diameter of the ligand and the complex particles Co(II), complex (4) was determined to be 39.25 ± 3.51 nm and 23.24 ± 2.45 nm respectively. All complexes are present in nano size particles i.e., their particles present in a diameter between 1 and 100 nm in size. The complex (4) and the ligand show signs with ratio that exhibit new or enhanced size-dependent properties compared with larger particles of the same material with many advantages such as:

Increased bioavailability, dose proportionality, decreased toxicity, smaller dosage form (i.e., smaller tablet), stable dosage forms of drugs which are either unstable or have unacceptably low bioavailability in non-nanoparticulate dosage forms, increased active agent surface area results in a faster dissolution of the active agent in an aqueous environment, such as the human body, faster dissolution generally equates with greater bioavailability, smaller drug doses, less toxicity and reduction in fed/fasted variability.

Table 4. ESR data for some metal (II) complexes:

<table>
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<tr>
<th>No.</th>
<th></th>
<th></th>
<th>(g_{iso})</th>
<th>(g_{ax})</th>
<th>(A_1)</th>
<th>(A_{2B})</th>
<th>(G)</th>
<th>(\Delta E_{ax})</th>
<th>(\Delta E_{sd})</th>
<th>(K_2)</th>
<th>(K_2)</th>
<th>(K_2)</th>
<th>(\beta/\alpha)</th>
<th>(\beta^2)</th>
<th>(\beta^2)</th>
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<th>(a_{2B}(%))</th>
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<td>222</td>
<td>0.43</td>
<td>1.13</td>
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</tr>
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</table>

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*Egypt. J. Chem. 64, No. 7 (2021)*
\( g_{\text{iso}} = \frac{2g_\perp + g_\parallel}{3} \), b) \( A_{\text{iso}} = \frac{2A_\perp + A_\parallel}{3} \), c) \( G = \frac{(g_\parallel - 2)}{(g_\perp - 2)} \)

Fig. 3. TEM images for ligand nanoparticles

Fig. 4. TEM images for Co(II) complex (4) nanoparticles
Cytotoxic Activity: Chemotherapeutic studies: The biological activity of the ligand (1) and its metal complexes (4), (8), and (12) were evaluated against MCF-7 cell line. In this study, we try to know the chemotherapeutic activity of the tested complexes by comparing them with the standard drug (IMURAN (azathioprine)). The treatment of the different complexes in DMSO showed similar effect in the tumoral cell line used as it was previously reported. The solvent dimethyl sulfoxide (DMSO) shows no effect in cell growth. The ligand (1) shows a weak inhibition effect at ranges of concentrations used, however, the complexes showed better effect against MCF-7 cell line. The obtained data indicate the surviving fraction ratio against MCF-7 cell line increasing with the decrease of the concentration in the range of the tested concentrations. Also, the Co(II) complex (4) shows the highest potency of inhibition at 50 µg/ml against MCF-7 cell lines, compared with the standard drug. Cytotoxicity results indicated that the tested complexes (4), (8) and (12) (IC$_{50}$ = 23.42–178.54 µM) demonstrated potent cytotoxicity against MCF-7 cancer cell as shown at Figures 5-9.

Fig. 5. Positive control of MCF-7 cells
Fig. 6. Effect of ligand (1) on MCF-7 cells at different concentration

Fig. 7. Effect of complex (4) on MCF-7 cells at different concentration
Fig. 8. Effect of complex (8) on MCF-7 cells at different concentration
From histograms we found that:

(1) Decrease in the number of available cells.
(2) Most of the remaining observed degeneration changes in the form of the irregularly cell membrane opaque and not well formed chromatin regulated of swelling cytoplasm, other showed optimistic change in the formed of shrunken cells and increase eosinophilia cells, and picknitoic nucleus.

**Conclusion**
In the present study, new metal(II) complexes of N-(2-((E) (2hydroxybenzylidene)amino) phenyl)-2-((E)-((6-hydroxycyclohexa-1,5-dien-1-yl)methylene) amino) butanamide were prepared. Structural and spectroscopic properties revealed that, the ligand adopted a triradentate or hexadentate ligand fashion; on the other hand, the metal complexes adopted a tetragonal distorted octahedral geometry around metal ions. All the complexes are non-electrolytic in nature as suggested by molar conductance measurements. The ligand coordinated to the central metal ion forming five six membered rings including the metal ions. The antitumor activities of the ligand as well as some of its metal complexes were assessed that, the toxicity of both ligand and some of its metal complexes was found to be concentration dependent, the cell viability decreased with increasing the concentration of complexes.

**References**