FULL PAPER



Characterization and thermal studies of nano-synthesized Mn(II), Co(II), Ni(II) and Cu(II) complexes with adipohydrazone ligand as new promising antimicrobial and antitumor agents

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complexes of N_1, N_6 -bis((2-hydroxynaphthalin-1-yl)methinyl)) New seven adipohydrazone (H₂L) with $MnCl_2 \bullet 4H_2O$, $CoCl_2 \bullet 6H_2O$, $NiCl_2 \bullet 6H_2O$, CuCl₂•2H₂O, Cu(NO₃)₂•3H₂O, CuSO₄•5H₂O, and Cu(OAc)₂•2H₂O have been prepared and characterized by the aid of elemental and thermal analyses, spectra (FT-IR, ¹H NMR, MS, UV-Vis, ESR, X-ray powder diffraction), molar conductance and magnetic moment measurements. The XRD results unambiguously confirmed the nano-sized particles of the complexes. The results showed that H₂L behaves as dibasic tetra-dentate ligand towards the metal ions of interest. The low molar conductance values revealed the non-electrolytic nature for the chelates. The magnetic moment data, UV-Vis and ESR spectra denoted the formation of octahedral geometries for Mn(II) and Ni(II) complexes, whereas Co(II), Cu(II) complexes exhibited tetrahedral arrangement. The activation parameters for the thermal decomposition stages were calculated from TGA curves using Coats-Redfern and Horowitz-Metzger methods. The obtained data were confirmed by 3-D molecular modeling of the ligand and some complexes. The investigated compounds were screened for their antimicrobial activities against different types of organisms and antitumor activities towards human liver Carcinoma (HEPG2) cell to access their potential chemotherapeutic use. The free ligand (H_2L) exhibited a weak inhibition of cell viability with IC_{50} of 11.80 µg/ml, complexes 4, 6 and 7 showed a moderate activity with IC₅₀ of 5.56, 7.71 and 5.67 μ g/ml, whereas complexes 1, 2, 3, and 5 displayed a strong anticancer activity with IC_{50} of 4.65, 3.97, 3.30 and 4.84 μ g/ml, compared with IC₅₀ value of 4.73 μ g/ml for the doxorubicin (standard cytotoxin drug).

KEYWORDS

antimicrobial, antitumor, hydrazone derivatives, nano-metal complexes, spectral

1 | **INTRODUCTION**

Adipodihydrazide is a symmetrical molecule with a two $CONHNH_2$ as reactive groups and C4 backbone. It offers amido- and amino functionality at both ends of the molecules which makes it splendid cross-linking agent for water based

emulsions. It is used for preparation of active forms of primarily neutral mannans from pathogenic yeasts (*Candida glabrata, Candida tropicalis, Candida albicans*). Exalted cross-linking influence of ADH was detected, despite the high surplus of ADH involved in the reaction.^[11] Also, it is used as an excellent hardener for certain epoxy resins, in

coating, adhesive and other manufacturing applications.^[2] Schiff bases are a serious category of compounds reported to own nitrogen carbon double bond which is answerable for various biological activities,^[3] including anticancer,^[4] antipyretic,^[5] antitumor,^[6] anti-inflammatory activities.^[7] They possess chelatogenic properties, so form interesting ligands in coordination chemistry.^[8-10] Transition metal complexes of hydrazones have many applications such as antibacterial, antifungal, antiviral, and anticancer agents and they are considered as important model for several biological systems.^[11] Hydrazones and their complexes accentuate a multilateral class of compounds in organic chemistry. These compounds have engaging biological properties such as antitumor, anti-inflammatory, antituberculous, analgesic, antimicrobial and anticonvulsant activity.^[12,13] Hydrazones are interesting compounds for drug design and the syntheses of heterocyclic compounds, organo-catalysis and also as possible ligands in metal complexes.^[14] Manganese is a substantial element for human health, essential for antioxidant system, metabolism, and development. The classes of enzymes that have manganese cofactors are large and involves transferases, oxidoreductases, lyases, hydrolases, isomerases, integrins, lectins and ligases.^[15] Cobalt is an important element to the metabolism of all animals. It is a key constituent of cobalamin, also known as vitamin B_{12} , the primary biological reservoir of cobalt as an ultratrace element. Proteins depend on cobalamin apply corrin to grasp the cobalt.^[16] Nickel plays an essential role in the biology of many plants, fungi, archaebacteria, and eubacteria. Enzymes of nickel such as urease are considered virulence factors in many organisms. Urease catalyses the hydrolysis of urea to compose carbamate and ammonia. The [NiFe]-hydrogenases catalyses the oxidation of H₂ to form electrons and protons, also it catalyzes the reverse reaction, formation of hydrogen gas through the reduction of protons.^[17] Copper is biostatic, touching of copper-alloy surfaces have natural properties which destroy a floppy range of microorganisms (e.g., adenovirus, influenza A virus, fungi, methicillin-resistant Staphylococcus aureus (MRSA), E. coli O157:H7, Staphylococcus and *Clostridium difficile*). Copper proteins have diversified roles in biological oxygen transportation and electron transport, processes that take advantage of the cushy interconversion of Cu(I) and Cu(II).^[18] Also, it has been reported that different salts of the same metal with the ligand gives complexes of different stereochemistry.^[19,20] Hence, our goal was to synthesize a new series of bioactive nano-sized Mn(II), Co(II), Ni(II) and Cu(II) chelates with N₁,N₆-bis(2hydroxynaphthalin-1-yl)methinyl)adipohydrazone for pharmaceutical applications. The subsistence of metal chelates in nanosized form expedites the absorption of such compounds by the human body and displays a towering efficiency of their vitality in treatment of diversified human diseases. The structural formulae of the complexes are

investigated using analytical and spectral techniques. The molecular modeling was performed for the ligand and some complexes to assert their structural formula.

2 | EXPERIMENTAL

Adipodihydrazide, hydrazine hydrate, triethylamine, ethanol, diethylether, DMSO, DMF, $MnCl_2 \bullet 4H_2O$, $CoCl_2 \bullet 6H_2O$, $NiCl_2 \bullet 6H_2O$, $CuCl_2 \bullet 2H_2O$, $Cu(NO_3)_2 \bullet 3H_2O$, $CuSO_4 \bullet 5H_2O$, and $Cu(OAc)_2 \bullet 2H_2O$ were of analytical grade and used without further purification.

2.1 | Synthesis of the organic ligand

Ligand N₁,N6-bis((2-hydroxynaphthalin-1-yl)methinyl)) adipohydrazone (H₂L) was synthesized using a procedure the literature,^[21] by condensation reported in of adipodihydrazide (1 mM, 0.1742 g) and 2-hydroxy-1naphthaldehyde (2 mM, 0.34436 g) in ethanol (30 mL). The reaction mixture was refluxed on a water bath for 20 h, with constant stirring in presence of four drops of acetic acid. The mixture was cooled to room temperature and the precipitated product was filtered off and was recrystallized from ethanol in order to get a pure sample. The scheme for the preparation of the free ligand (H₂L) is presented in Scheme 1. The purity of ligand was checked by TLC and melting point constancy.

2.2 | Synthesis of the nano-sized metal complexes

The nano-sized metal complexes were synthesized by mixing hot ethanolic solution of 1 mM (0.4820 g) of ligand (H₂L) with 1 mM of the metals salts [MnCl₂•4H₂O (0.1979 g), CoCl₂•6H₂O (0.2379 g), NiCl₂•6H₂O (0.2377 g), CuCl₂•2H₂O (0.1705 g), Cu(NO₃)₂•3H₂O (0.2416 g), CuSO₄•5H₂O (0.2379 g), and Cu(OAc)₂•2H₂O (0.2497 g)] dissolved in ethanol (40 ml) in the presence of five drops of triethylamine as basic medium. The resulting solution was



SCHEME 1 Preparation of N_1 ,N6-bis((2-hydroxynaphthalin-1-yl) methinyl))adipohydrazone (H₂L)

gently refluxed for 18 h. Both contentious heating and the presence of triethylamine as basic medium enhance the formation of nano-sized particles. After cooling, the resulting precipitate was filtered off, washed several times with ethanol and finally dried in a vacuum dessicator over anhydrous CaCl₂. The scheme for synthesis of nano-sized metal complexes (1–7) is given in Scheme 2. The solids complexes were subjected to different analytical and spectroscopic tools.

2.3 | Analysis and instrumentations

C, H and N contents were determined micro-analytically at the Micro-Analytical Center, Cairo University by Perkin-Elmer 2400 CHN Elemental Analyzer. The metal content was determined using inductively coupled plasma (ICP) technique after decomposition of the metal complexes using nitric acid. The molar conductivities of the metal complexes were measured in DMF $(10^{-3} \text{ mol } \text{L}^{-1})$ by the aid of a conductivity meter JENWAY model 4070 Conductance Bridge at room temperature. Infrared spectra of the ligand and its metal complexes in KBr disks were recorded on a Jasco FT-IR-4100 (made in Japan) within 4000-200 cm⁻¹ range at Central Laboratory, Tanta University, Egypt.¹HNMR spectra were investigated using a Mercury Oxford NMR 300 Hz spectrophotometer using tetramethylsilane (TMS) as internal standard after dissolving the samples in DMSO- d⁶ (EI-MS). Standard electron impact mass spectra (E.I) of the free ligand and its metal complexes were performed by a Finnigan MAT 8222 Spectrometer at 70 eV at Micro-Analytical Unit of Cairo University. Electronic absorption spectra were taken in freshly prepared DMF solution from 200 to 800 nm with



SCHEME 2 Synthesis of synthesis of nano-sized metal complexes (1-7), where MX₂ is Mn(II), co(II), Ni(II), and cu(II) with different anions

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T80 + UV-Vis spectrometer. Room temperature magnetic susceptibilities were measured using a Sherwood scientific magnetic susceptibility balance. Experimental magnetic susceptibility values have been corrected for diamagnetism of the constituent atoms by the use of Pascal's constants.^[22] EPR spectra of some Cu(II) complexes as powders were obtained on a Jeol JES-RE1X EPR spectrometer working in the X-band (9.435 GHz). A spectrum was obtained in a 2-mm quartz capillary at room temperature at Alexandria University. XRD patterns of the samples were recorded on X-ray diffractometer (GNR, APD2000PRO, Italy) at Central Laboratory, Tanta University, Egypt. All patterns were obtained using Cu/K α_1 radiation with a graphite monochromator at 0.03° min⁻¹ scanning rate. The thermal gravimetric analysis (TGA) of the solid complexes was measured via a Shimadzu TG-50 thermal analyser from ambient temperature up to 800°C under nitrogen as atmosphere with 10°C/min heating rate.

2.4 | Antimicrobial activity (sensitivity) tests

Antimicrobial activity of the free ligand and its metal complexes was tested using a modified Kirby-Bauer disc diffusion method,^[23] at Micro-Analytical Center, Cairo University. Standard discs of Ampicillin (antibacterial agent), Norfloxacin (antibacterial agent) and Amphotericin B (antifungal agent) served as positive controls but filter discs impregnated with 10 µL of solvent (distilled water, chloroform, DMSO) were used as a negative control. 100 µL of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of 108 cells/ml for bacteria and 105 cells/ml for fungi,^[24] approximately. 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were preserved. Isolated colonies of each organism that might be playing a pathogenic role should be elected from primary agar plates and tested for susceptibility by disc diffusion method.^[25] NCCLS recommends Mueller-Hinton agar of the many media available, because it results in good batch-to-batch reproducibility. For evaluating the susceptibilities of filamentous fungi to antifungal agents, disc diffusion method for filamentous fungi tested by using approved standard method (M38-A) developed by the NCCLS, 2002.^[26] Disc diffusion method for yeasts expanded by using approved standard method (M44-P) by the NCCLS, 2003.^[27] Plates inoculated with filamentous fungi as Aspergillus flavus at 25 °C for 48 h; Gram (-) bacteria as, Escherichia coli; Gram (+) bacteria as Staphylococcus aureus; they were incubated at 35-37 °C for 24-48 h and yeast as Candida albicans incubated at 30 °C for 24-48 h and, then the diameters of the inhibition zones were measured in millimetres.^[23] Meuller-Hinton agar is used as agar that is rigorously tested for composition and pH. Furthermore, the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been measured for susceptible and resistant values. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8.0 mm were impregnated 10 µL of tested concentration of the stock solutions. The chemical will diffuse from the disc into the agar, when a filter paper disc impregnated with a tested chemical is placed on agar. This diffusion will place the chemical in the agar only around the disc. The molecular size of the chemical and its solubility will determine the size of the area of chemical infiltration around the disc. If an organism is placed on the agar it will not grow in the area around the disc if it is susceptible to the tested material. This area of no growth around the disc is known as a clear zone or zone of inhibition. The zone diameters were measured with slipping calipers of the National Committee for Clinical Laboratory Standards,^[28] for the disc diffusion. Agar-based methods such as E-test and disk diffusion can be good alternatives because they are faster and simpler than brothbased methods.^[29,30]

2.5 | Potential cytotoxicity measurements

2.5.1 | Cell line

The cell lines were obtained from the American type culture collection (ATCC, Rockville, MD). The cells were maintained at 37 °C in humidified atmosphere with 5% CO₂ and were sub-cultured two to three times a week. The cells were grown as mono layers on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 μ g/L gentamycin.

2.5.2 | Evaluation of the antitumor activity

The antitumor activity was screened on Carcinoma cell lines at the Regional Center for Microbiology and Biotechnology, Al-Azhar University, Cairo, Egypt. The minelayers of 10.000 cells adhered at the bottom of the wells in a 96-wellmicrotiter plate Falcon, NJ, USA) incubated at 37 °C for 24 h., in a humidified incubator with 5% CO₂. The monolayer was then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 μ L from different dilutions of tested sample in fresh maintenance medium and incubated at 37 °C. A control of untreated cells was made in the absence of the tested sample. Three wells were used from each concentration of the test sample. Every 24 h, the observation was made under inverted microscope. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid. After well mixing, read the absorbance at 590 nm using ELISA reader. The absorbance values from untreated cells were considered as 100% proliferation and the percentage of viability was calculated as (1-(ODT/ODC)*100% where ODC is the mean optical density of untreated cells and ODT is the mean optical density of the cells treated with the tested sample.^[31–33]

3 | RESULTS AND DISCUSSION

3.1 | Stoichiometry and nature of the complexes

The ligand and its metal complexes with their molecular and empirical formulae, molecular weight, colour, melting point, conductance and analytical data are compiled in Table 1. All complexes (1-7) are air-stable, coloured, insoluble in water and most common organic solvents such as, ethanol, methanol, acetone but they dissolve in DMF and DMSO. The intense colour of the complexes can be attributed to a charge transfer or a defect of crystal.^[34] The analytical data prove that H₂L forms complexes which agree well with 1:1 metal to ligand stiochiometry with MnCl₂•4H₂O, CoCl₂•6H₂O, NiCl₂ \bullet 6H₂O, $Cu(NO_3)_2 \bullet 3H_2O$, $CuSO_4 \bullet 5H_2O$, and Cu(OAc)₂•2H₂O, but forms complex within 1:2 metal to ligand stiochiometry with CuCl₂•2H₂O. The yield for the synthesized complexes was 75.5-90.4%. The molecular formulae of all the complexes were confirmed by spectral data. The measured molar conductance values for the complexes lie within 6.80–12.70 ohm^{-1} cm² mol⁻¹ range (Table 1). These values indicate their non-electrolytic nature.^[35]

3.2 | ¹H NMR and FT-IR spectra

¹H NMR spectrum of H₂L displayed a signal at 12.6 ppm can be attributed to δOH . In comparison, the stretching vibration band of OH (ν OH) appeared at 3475 cm⁻¹ in IR spectrum of H_2L . The -CH = N- proton appeared as a singlet at 9.2 ppm in ¹H NMR spectrum of the ligand. This is supported by appearance of $\nu C = N$ band appeared at 1620 cm⁻¹ in its IR spectrum.^[36] The multiplet signals that appeared at 7.2–7.6 ppm are assignable to aromatic protons.^[37] FT-IR spectra of Mn(II), Co(II) and Ni(II) and Cu(II) complexes (1-7) were compared with that of H₂L in order to elucidate the mode of bonding in the formed complexes (Table 2). $\nu C = N$ band appeared at 1620 cm⁻¹ in H₂L spectrum, displayed average shift to lower frequency by 3-22 cm⁻¹ indicating coordination of C = N group to the metal centre.^[19] H₂L spectrum exhibited a strong band at 1659 cm⁻¹ due to $\nu C = O$ which confirm that H₂L is stable in its ketoform. This band has no or a slight small average frequency shifts in case of metal complexes (1-7) ruling out the possibility of coordination of carbonyl oxygen atom to the metal centre.^[34] This originates from coordination of both the naphtholate oxygen atoms and azomethine nitrogen atoms of the same ligand molecule to the same metal centre.

TABLE 1 Physical characteristics, microanalysis, and molar conductance data of H₂L and its metal complexes (1-7)

	Molecular formula	M Wt Found	Colour		Microanalysis Found (Calc.) %			
Comp. No.	(Empirical formulae)	(Calc.)	$(\Lambda_{\rm m})$	%C	% H	%N	%M	
Ligand	$H_{2}L$ (C ₂₈ H ₂₆ N ₄ O ₄)	482.21 (482.54)	Milky ()	70.00 (69.70)	4.81 (5.43)	11.28 (11.61)		
Complex 1	$[MnL] \bullet H_2O$ $(C_{28}H_{26}MnN_4O_5)$	553.49 (553.47)	brown (8.2)	61.02 (60.76)	5.19 (4.73)	10.22 (10.12)	9.94 (9.93)	
Complex 2	$[CoL] \bullet C_2 H_5 OH$ $(C_{30} H_{30} CoN_4 O_5)$	585.11 (585.52)	D. brown (12.7)	61.98 (61.54)	6.87 (5.16)	9.72 (9.57)	10.00 (10.07)	
Complex 3	$[NiL] \bullet C_2 H_5 OH$ $(C_{30}H_{30}N_4 NiO_5)$	585.51 (585.28)	F. yellow (6.8)	62.62 (61.57)	5.74 (5.17)	9.65 (9.57)	10.03 (10.03)	
Complex 4	$[CuL_{2}]\bullet C_{2}H_{5}OH \\ (C_{58}H_{56}CuN_{8}O_{9})$	1071.00 (1072.67)	Green (7.5)	66.37 (64.94)	5.46 (5.26)	10.44 (10.95)	5.42 (5.92)	
Complex 5	$\label{eq:cul} \begin{split} & [CuL] \bullet H_2O \\ & (C_{28}H_{26}CuN_4O_5) \end{split}$	562.01 (562.08)	Green (10.3)	58.79 (59.83)	4.43 (4.66)	9.81 (9.97)	11.18 (11.30)	
Complex 6	$[CuL] \bullet 2H_2O \\ (C_{28}H_{28}CuN_4O_6)$	580.10 (580.09)	Green (11.5)	57.29 (57.98)	4.55 (4.86)	9.65 (9.66)	10.50 (10.95)	
Complex 7	$[CuL] \bullet \frac{1}{2}H_2O \\ (C_{28}H_{25.5}CuN_4O_{4.5})$	553.74 (553.07)	Green (9.5)	60.37 (60.80)	3.82 (4.65)	10.56 (10.13)	11.41 (11.45)	

M. Wt is the molecular weight obtained from mass spectra and Λ_m is the molar conductance (Ω^{-1} cm² mol⁻¹)

This should introduce steric crowding in the complex molecule. So, one dihydrazone arm remains in the equatorial plane while the other hydrazone arm attains axial position.^[38] Complexes **1**–7 displayed broad bands within 3450–3424 cm⁻¹ range, which can be attributed to ν (OH) of water or ethanol molecules associated with complexes. The presence of water and ethanol molecules renders it difficult to confirm the deprotonation of the OH groups on complex formation from the stretching vibration.^[39] All these observations are supported by appearance of new non-ligand bands at 655–638 and 546–531 due to ν (M–O) and ν (M–N), respectively.^[36]

TABLE 2 Assignments of IR spectral bands (cm^{-1}) of diagnostic importance for the free ligand (H₂L) and its metal complexes (1-7).

Compound	ν_{OH}	$\nu_{\rm NH}$	$v_{C=0}$	$\nu_{C=N}$	$\nu_{\text{C-O}}$	$\nu_{\text{M-O}}$	$\nu_{\text{M-N}}$
H_2L	3475	3045	1659	1620	1320		
Complex 1	3424	3047	1671	1607	1347	655	534
Complex 2	3432	3051	1658	1610	1329	653	546
Complex 3	3450	3041	1668	1608	1348	651	531
Complex 4	3444	3046	1662	1609	1334	649	540
Complex 5	3434	3048	1659	1617	1335	640	538
Complex 6	3457	3043	1659	1610	1348	650	539
Complex 7	3439	3050	1660	1598	1334	638	544

3.3 | Mass spectral analysis

Mass spectra are used to confirm the constitutions and purities of the prepared ligand and its chelates (Figure 1). The free ligand fragmentation exhibits the molecular ion peak (calc.) at 482.21 (482.53), which is matched with its theoretical value. Mass spectra of [MnL] \bullet H₂O, [CoL] \bullet C₂H₅OH, [NiL] \bullet C₂H₅OH, [CuL] \bullet H₂O, [CuL] \bullet H₂O and [CuL] \bullet '/₂H₂O displayed accurate molecular ion peaks (calc.) at 553.49 (553.47), 585.11 (585.52), 585.51 (585..28), 562.01 (562.08), 580.10 (580.09), 553.74 (553.07), respectively, corresponding to the parent ion [ML]⁺. Also,

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FIGURE 1 Mass spectrum of [CuL]•H₂O (5)

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appearance of different peaks due to various fragments during successive degradation of the target compound is a perfect support for the suggested molecular structure.^[40] New idealistic evidence support the proposed structure of the complexes comes from the decomposition of metal complexes (1–7) via abstraction of H₂L, which give rise to the presence of a molecular ion peak attributable to the free ligand. This is prevalent demeanour for metal chelates containing different ligands (ML) that decompose through cleavage of the metal–ligand bond during the ionization process.^[41]

3.4 | Electronic spectra and magnetic moment measurements

The UV–Vis spectrum of the free ligand (H₂L) showed three bands in DMF at 270, 324 and 372 nm which can be assigned to π — π^* transition of the aromatic moiety, π — π^* transition of CH = N group and charge transfer (CT) interaction within the whole molecule. These bands displayed obvious shifts in the spectra of metal complexes supporting the coordination of the ligand to the central metal ions.^[42] The electronic spectrum of Mn(II) complex showed two non-ligand bands at 436 and 530 nm assignable to ${}^{6}A_{1g} \rightarrow {}^{4}E_{g}$ and ${}^{6}A_{1g} \rightarrow {}^{4}T_{2g}$ transitions, respectively, in an octahedral configuration.^[43] The observed magnetic moment value of Mn(II) complex equals 5.67 BM (Table 3). denotes Mn(II) complex is in the high-spin state in an octahedral geometry.^[44] Co(II) complex displayed two electronic absorption bands at 436 and 625 nm due to ${}^4A_2 \rightarrow {}^4$ $T_1(P)$ and ${}^4A_2 \rightarrow {}^4$ $T_1(F) transitions, respec$ tively, in a tetrahedral arrangement.^[45] Co(II) complex gave magnetic moment value (4.36 BM) which is higher than theoretical spin only value referring to orbital contribution of Co(II) complex.^[46] The UV-Vis spectrum of Ni(II)

TABLE 3 UV-Vis, ESR spectral data and magnetic moment valuesof the free ligand (H_2L) and metal complexes (1-7).

	Electronic (λ _{max} ; nm	c spectra) in DMF		
Compound	Ligand bands	Non-ligand bands	μ_{eff} (magnetic moment; BM)	g _{eff} _values
Ligand	270, 324, 372	—, —	—	_
Complex 1	268, 332, 412	436, 530	5.67	_
Complex 2	288, 318, 336	436, 625	4.36	—
Complex 3	272, 326, 370	438, 550	3.16	_
Complex4	276, 324, 374	438, 545	2.10	2.1380
Complex 5	275, 332, 374	440, 600	1.72	2.1240
Complex 6	274, 324, 372	430, 570	2.00	2.1189
Complex 7	276, 344, 372	435, 580	2.03	2.1222

complex showed two bands in DMF at 438 and 550 nm, corresponding to ${}^{3}A_{2g} \rightarrow {}^{3}T_{2g}(P)$ and ${}^{3}A_{2g} \rightarrow {}^{3}T_{2g}(F)$ transitions, respectively, in an octahedral geometry.^[47] Ni(II) complex displayed magnetic moment value equals 3.16 BM supporting the octahedral environment around Ni(II) center.^[48] The electronic spectra of Cu(II) complexes (4–7) displayed two new bands within 430–440 and 545–600 nm ranges which can be attributed to ${}^{2}T_{2} \rightarrow {}^{2}E_{2}$ and charge transfer transitions in tetrahedral Cu(II) complexes.^[49] The subnormal μ_{eff} value of Cu(II) complexes (1.72–2.10 BM) in some cases reveals the Cu-Cu interaction.^[50]

3.5 | ESR spectra of cu(II) complexes

The X-band ESR spectra of the powder Cu(II) complexes (4–7) at room temperature showed a broadened feature without hyperfine splitting due to the dipolar interaction from the ESR spectrum of a set of magnetic parameters. Two anisotropic signals were observed in ESR spectra of all Cu(II) complexes under interest (Figure 2). The profile of ESR spectra is characteristic of tetrahedral geometry around Cu(II) center which support and agree with the data obtained from electronic spectral results. The g_{eff}-values (2.1189–2.1380) of Cu(II) complexes (4–7) have a positive contribution from the value of the free electron (2.0023) due to the measurable covalent character in the bonding between the ligand and Cu(II) ion. So, we can conclude that the metal ligand bonding in these complexes has essentially covalent character.^[51]



FIGURE 2 X-band ESR spectrum of cu(II) complex (6)

3.6 | Thermo-gravimetric analysis

The TGA-DTG curves of the metal complexes under investigation were recorded from ambient temperature up to 800 °C at heating rate of 10 °C/min under N2 gas flow as shown in (Figure 3). From TGA curves, the mass loss was determined for the different thermal decomposition steps and compared with those theoretically calculated for the suggested formula based on the data of elemental analyses. In most cases, TGA denoted the formation of metal oxide as the final product from which the metal content is calculated and found to be in satisfying agreement with that acquired from analytical measurements.^[52] The obtained data revealed that all metal chelates decomposed in two steps for complex (4), four steps for 1, 3, 5 and 7 and five steps for 2 and 6. The stages of decomposition, temperature ranges, theoretical calculated, and found mass losses as well as final products observed in each step are given in Table 4.

3.7 | Thermodynamic and kinetic parameters

The mechanism, activation energy (*E*), reaction order (n) and, frequency factor (*A*), for different stages of thermal decomposition of the complexes (1–7) were determined applying integral method proposed by Coats-Redfern (Figure 4).^[53] and the approximation method proposed by Horowitz– Metzger.^[54] The thermodynamic activation parameters namely, enthalpy (ΔH^*), entropy (ΔS^*) and free energy change (ΔG^*) of different decomposition steps were calculated applying the following relationships.^[55]

$$\Delta H^* = E^* - RT \tag{1}$$

$$\Delta S^* = R[\ln(Ah/K_BT) - 1] \tag{2}$$

$$\Delta G^* = \Delta H^* - T \Delta S^* \tag{3}$$

Where T is the observed peak temperature, ΔH^* is the activation enthalpy (kJ mol⁻¹), ΔS^* is the activation entropy



FIGURE 3 TGA curves of complexes 1, 3 and 5

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(J mol⁻¹ k⁻¹), ΔG^* is the Gibbs free energy (kJ mol⁻¹), K is the Boltzmann constant and h is the Plank constant.

The kinetic and thermodynamic activation parameters of thermal decomposition stages of the metal complexes are given in Tables 5 and 6. Δ H* values are positive which reveals that the decomposition processes are endothermic. ΔS^* values for complexes were found to be positive. This reveals that the activated complex is less ordered than the reactants and/or the reactions are fast.^[56] ΔG^* values increase in a sufficiently great or important way for the subsequently decomposition stages due to increasing $T\Delta S^*$ values of one step to another which override ΔH^* values. This reflects that the rate of removal of the subsequent ligand parts will be lower than that of the precedent.^[57] This can be assigned to the structural rigidity of the remaining complex after the expulsion of one or more ligand part, as compared with the precedent complex, which requires more energy for its rearrangement before undergoing any compositional change.

3.8 | XRD studies

Serious structural information about microcrystalline samples of H₂L and its metal complexes can be provided by XRD (powder technique).^[58] The diffraction patterns were recorded within 20 (scattering angle) from 10 to 90°. XRD patterns of free ligand H₂L are different compared with those of each metal complex (Figures 5 and 6). Also, the inter-planar spacing d (Å) and relative intensities (I/I_0) of each compound are different from each other. The XRD patterns declare crystalline nature for the compounds under investigation. This behaviour may probably be due to the incorporation of the water or ethanol molecules. A comparative study in the patterns indicates the absence of smearing or contamination with starting materials. A shift in diffraction peaks of complexes was observed, suggesting a contribution of the ligand groups. All these observations support the formation of single phase metal complexes. The inter-planar distance d (the volume average of the crystal dimension normal to diffracting plane) is calculated from 2θ values applying Bragg's law, which describes the condition on θ for the constructive interference to be at its strongest:^[59]

$2d\sin\theta = n\lambda$,

where, n is a positive integer and λ is the wavelength of incident wave. The values of θ , *d*, β [the line broadening at half the maximum intensity (FWHM)] of prominent intensity peak, and particle size of the investigated compounds are given in Table 7. The size of particle or crystallite size was calculated from XRD patterns by applying the Scherrer equation.^[60] It is a formula that relates the size of sub-micrometre particles, or crystallites, in a solid to the broadening of a peak in a diffraction pattern, in X-ray diffraction and

TABLE 4 Thermo-gravimetric analysis (TGA) of the metal complexes (1-7).

Molecular	Temp. range	Mass loss %		Temp. range Mass loss %		
formula	(°C)	Found	Calc.	Assignment		
[MnL]•H ₂ O (1)	35-95	3.15	3.25	Loss of hydrated H ₂ O molecule.		
	95-290	10.12	10.11	Removal of (-CH ₂ -CH ₂ -CH ₂ -CH ₂ -)		
	290-350	15.5	15.53	Elimination of 2(C=O), (N ₂ H ₂).		
	350-590	61.10	61.17	Decomposition of the organic ligand and formation of MnO as final product.		
[CoL]•C ₂ H ₅ OH (2)	25-125	7.50	7.85	Loss of C_2H_5OH molecule.		
	125-200	14.11	14.34	Removal of (-CH ₂ -CH ₂ -CH ₂ -CH ₂ -), C=O.		
	200-300	14.80	14.68	Elimination of (C=O), (N ₄ H ₂).		
	300-360	14.30	14.00	Elimination of C ₂ H ₂ , 2(C-O).		
	360-560	39.00	39.04	Decomposition of the organic ligand and formation of CoO as final product.		
[NiL]•C ₂ H ₅ OH (3)	32-95	7.25	7.86	Loss of C_2H_5OH molecule.		
	95-295	14.00	14.35	Removal of (-CH ₂ -CH ₂ -CH ₂ -CH ₂ -), C=O.		
	295-365	14.82	14.69	Elimination of C=O, N ₂ H ₂ , 2N.		
	365-420	52.90	53.07	Decomposition of the organic ligand with formation of NiO as final product.		
[CuL ₂]●C ₂ H ₅ OH (4)	40-140	3.82	4.28	Loss of C_2H_5OH molecule.		
	140-400	80.12	80.54	Decomposition of the organic ligand with formation of CuO + 8C		
[CuL]●H ₂ O (5)	50-125	3.04	3.20	Loss of hydrated H ₂ O molecule.		
	125-270	14.34	14.94	Removal of (-CH ₂ -CH ₂ -CH ₂ -CH ₂ -), C=O.		
	270-355	29.50	29.88	Elimination of C=O, N ₂ H ₂ , 2N, 2(C-O).		
	355-470	40.50	40.66	Decomposition and formation of Cu as final product.		
[CuL]●2H ₂ O (6)	30-118	6.18	6.20	Loss of hydrated 2H ₂ O molecule.		
	118-193	9.27	9.65	Removal of (-CH ₂ -CH ₂ -CH ₂ -CH ₂ -).		
	193-293	5.15	5.17	Elimination of N ₂ H ₂ .		
	293-312	10.30	10.68	Elimination of 2C=O.		
	312-600	57.73	57.33	Decomposition of the organic ligand with formation of CuO as final product.		
[CuL]•½H ₂ O (7)	30-70	2.06	1.62	Loss of hydrated 1/2H ₂ O molecule.		
	70-230	15.46	15.18	Removal of (-CH ₂ -CH ₂ -CH ₂ -CH ₂ -), C=O.		
	230-360	11.34	10.88	Elimination of C=O, N ₂ H ₂ .		
	360-690	59.79	59.76	Decomposition of the organic ligand and formation of Cu as final product.		



FIGURE 4 Coats-Redfern plot for cu(II)-complex (6); (a) first step, (b) second step, (c) third step and (d) fourth step, where, $Y = [1-(1-\alpha)^{1-n}] / (1-n) T^2$ for $n \neq 1$ or $Y = [-\ln(1-\alpha)] / T^2$ for n = 1

crystallography.^[61] This equation is used to determine the size of particles of crystals in the form of powder. Scherrer equation can be written as:

$$\tau = \frac{K\lambda}{\beta\,\cos\theta}$$

where, λ is the x-ray wavelength, τ is the mean size (crystallite size) of the ordered (crystalline) domains, which may be smaller or equal to the grain size, *K* is a dimensionless shape factor, with a value close to unity (the shape factor has a typical value of about 0.9, but varies with the actual shape of the crystallite), β is the line broadening at half the maximum intensity (FWHM), after subtracting the instrumental line broadening, in radians (this quantity is also sometimes denoted as $\Delta(2\theta)$ and θ is the Bragg angle. The average particle size calculated from different diffraction peaks of H₂L, **1**, **2**, **3**, **4**, **5**, **6**, and **7**, compounds were found to be 31.26, 38.79, 19.88, 88.99, 6.21, 26.52, 7.98 and 18.38 nm, respectively.

3.9 | Molecular modeling studies

Molecular orbital geometry optimization allows a quantitative discussion of the geometrical properties of the inspected complexes. Geometric structures of H_2L and complexes (1, 2, 3 and 5) were computed by optimization of their bond

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TABLE 5 Thermodynamic and kinetic parameters of Mn(II), Co(II), Ni(II) and Cu(II) complexes (1-7) calculated from TGA curves using Coats-Redfern method.

Comp.	n	Step	r	T (K)	E *	Δ H*	Α	$-\Delta S^*$	ΔG^*
1	1	1 st	0.9991	338	19.50	15.31	3605689	0.1321	81.77
	1	2 nd	0.9984	434	8.67	1.23	8.41×10 ⁸	0.0923	67.72
	0.33	3 rd	0.9970	592	132.50	169.02	3.43×10 ⁻⁵	0.2742	336.22
	1	4 th	0.9998	779	57.98	55.25	6688068	0.1234	95.84
2	1	1^{st}	0.9866	333	52.31	49.65	53.27	0.2208	120.21
	0.66	2^{nd}	0.9938	425	162.58	158.67	5.64×10 ⁻¹⁰	0.4341	362.95
	0.5	3^{rd}	0.9978	498	87.73	84.42	39.82	0.2250	174.11
	1	4^{th}	0.9965	618	191.77	188.46	6.78×10 ⁻⁶	0.3546	329.80
	1	5^{th}	0.9966	718	102.16	97.61	3482.53	0.1905	201.79
3	1 1 1	1 st 2 nd 3 rd 4 th	0.9965 0.9966 0.9998 0.9999	315 437 605 663	64.75 20.82 488.68 136.09	59.66 15.73 481.35 133.36	0.2226 46005859 2.1×10 ⁻³ 1.53	0.2717 0.1125 0.8495 0.2505	225.79 84.52 1230.22 215.42
4	0	1 st	0.9929	379	9.77	4.32	1.31×10 ⁹	0.0852	60.18
	1	2 nd	0.9915	599	81.64	75.48	3669.33	0.1926	218.08
5	1	1 st	0.9991	326	95.62	92.89	1.17×10 ⁻⁶	0.3485	207.54
	0.66	2 nd	0.9993	477	80.301	74.91	58.56	0.2258	221.35
	0.66	3 rd	0.9999	577	132.47	129.15	0.09	0.2753	238.97
	0.33	4 th	0.9995	683	104.40	101.09	1278.714	0.1962	179.34
6	1 0.66 1 1 0.33	1^{st} 2^{nd} 3^{rd} 4^{th} 5^{th}	0.9907 0.9860 0.9990 0.9953 0.9999	334 421 470 570 777	26.13 46.63 163.59 257.03 23.94	23.37 43.87 160.82 254.27 21.18	623462.544629.183.91×10-85.09×10-136.81×108	0.1432 0.1651 0.3960 0.4895 0.0850	70.98 98.77 292.48 417.02 49.45
7	1	1 st	0.9428	315	41.72	38.95	978.26	0.1969	104.41
	0.5	2 nd	0.9921	448	30.34	27.58	4795514	0.1262	69.55
	0.5	3 rd	0.9872	586	43.64	40.87	4209989	0.1273	83.21
	1	4 th	0.9855	840	82.62	79.86	815686.60	0.1410	126.73

E*: the activation energy (kJ mol⁻¹), Δ H*: the activation enthalpy (kJ mol⁻¹), Δ S*: the entropy (J K⁻¹ mol⁻¹), A: the pre-exponential factor (s⁻¹), Δ G*: the free energy (KJ mol⁻¹).

lengths, bond angles and dihedral angles applying the hyper chem. 8.03 molecular modelling program.^[62] The optimized molecular structures with minimum energies obtained from the quantum chemical calculations are presented in Figures 7 and S1-S4. Selected bond angles and bond lengths are given in Tables S1-S5. As shown in Figure 7, the investigated ligand has a planner form. The bond lengths of atoms in the two chelation centres are N(35)-C(36) = 1.295, N(33)-C(34) = 1.317, N(30)-N(35) = 1.389, N(29)-N(33) = 1.397, C(26)-O(28) = 1.221, C(25)-O(27) = 1.218, C(26)-N(30) = 1.440,C(12)-O(37) = 1.366, C(25)-N(29) = 1.453 and C(8)-O(39) = 1.354 Å. Upon complexation, the bond lengths of the ligand molecules are somewhat altered, especially for atoms in direct contact with the metal ion centres. The other bond lengths within the ligand are slightly or not affected by the coordination to the metal ion. The calculations showed that Mn-complex attains an octahedral geometry which agrees with the proposed experimental octahedral geometry. The bond lengths of the chelating atoms with the central metal

atom are Mn(37)-O(39) = 1.891, Mn(37)-O(38) = 1.892, O(36)-Mn(37) = 1.904, O(35)-Mn(37) = 1.906, N(33)-Mn(37) = 1.908, N(31)-Mn(37) = 1.902 Å. Also, the guantum chemical calculations donated that Ni-complex has an octahedral geometry in good agreement with the obtained experimental results. The bond lengths of the chelating atoms with Ni atom are Ni(37)-O(39) = 1.872, Ni(37)-O(38) = 1.872, O(36)-Ni(37) = 1.884, O(35)-Ni(37) = 1.886,N(33)-Ni(37) = 1.889 and N(31)-Ni(37) = 1.882 Å. The bond angles around Mn and Ni atoms (Tables S2 and S4) are quite near to an octahedral arrangement,^[63] indicating sp³d² hybridization. Mn(II) and Ni(II) ions are coordinated to the ligand through the two N-atoms, oxygens of carbonyl groups and naphtholate O-atoms. Co(II) and Cu(II) complexes are found to be distorted tetrahedral structure where the two naphtholate O-atoms and two N-atoms atoms are coordinated to the metal center. The bond lengths between Co and chelating atoms are O(38)-Co(39) = 1.884, O(37)-Co(39) = 1.7517, N(35)-Co(39) = 1.901 and N(33)-Co(39) = 1.845 Å. In Cu(II)

TABLE 6 Thermodynamic and kinetic parameters of Mn(II), Co(II), Ni(II) and Cu(II) complexes (1-7) calculated from TGA curves using Horo-witz–Metzger method.

Comp.	n	Step	r	T (K)	E *	Δ H*	Α	-ΔS*	ΔG^*
1	2	1 th	0.927	337.5	17.470	14.876	146.73	0.203	78.465
	0	2 nd	0.964	434.25	1.495	1.098	0.041	0.271	87.149
	1	3 rd	0.920	592.5	1.552	1.041	0.024	0.275	85.884
	1	4 th	0.933	779.5	0.624	1.969	0.008	0.285	90.994
2	2	$1^{ m th}$	0.942	333	31.999	29.405	77486.08	0.151	76.731
	1	$2^{ m nd}$	0.963	425.5	4.209	1.615	0.197	0.258	82.346
	0	$3^{ m rd}$	0.935	498	1.220	1.373	0.024	0.276	87.564
	2	$4^{ m th}$	0.949	618	2.565	0.028	0.129	0.262	81.869
	2	$5^{ m th}$	0.947	718	0.604	1.989	0.009	0.284	90.639
3	2	1 th	0.998	314.75	95.804	93.210	9.37×10 ⁵	0.060	112.079
	2	2 nd	0.964	437	4.011	1.417	0.080	0.266	84.480
	0	3 rd	0.922	605.25	1.135	1.457	0.019	0.277	88.156
	0	4 th	0.918	663	0.559	2.034	0.009	0.283	90.552
4	2	1 th	0.935	378.75	5.149	2.555	0.171	0.259	83.652
	0	2 nd	0.927	603.5	0.521	2.072	0.010	0.283	90.487
5	1	1 th	0.934	325.5	28.242	25.648	8200.320	0.170	78.801
	0	2 nd	0.923	477	1.698	0.895	0.022	0.276	87.235
	2	3 rd	0.942	576.5	1.916	0.677	0.048	0.270	85.047
	2	4 th	0.928	682.5	1.769	0.824	0.034	0.273	86.078
6	2	$1^{ m th}$	0.982	334.12	29.301	26.707	9801.40	0.168	79.397
	1	$2^{ m nd}$	0.905	421	3.764	1.170	0.072	0.267	84.505
	0	$3^{ m rd}$	0.923	469.75	1.777	1.213	0.022	0.276	87.141
	2	$4^{ m th}$	0.921	569.87	2.464	0.129	0.059	0.268	83.978
	2	$5^{ m th}$	0.959	789.37	0.883	1.710	0.020	0.277	88.341
7	2	1^{th}	0.992	315.37	72.804	70.210	7.76×10 ¹¹	0.017	75.723
	0	2^{nd}	0.986	448	1.752	0.841	0.029	0.274	86.516
	2	3^{rd}	0.986	586.25	2.158	0.435	0.057	0.269	84.375
	0	4^{th}	0.955	840.25	0.222	2.371	0.005	0.289	92.587

E*: the activation energy (kJ mol⁻¹), Δ H*: the activation enthalpy (kJ mol⁻¹), Δ S*: the entropy (J K⁻¹ mol⁻¹), A: the pre-exponential factor (s⁻¹), Δ G*: the free energy (KJ mol⁻¹).



FIGURE 5 XRD spectral patterns of the free ligand (H_2L)



FIGURE 6 XRD spectral patterns of co(II) complex 2

complex (5), the bond lengths between Cu and chelating atoms are O(38)-Cu(39) = 1.893, O(37)-Cu(39) = 1.893, N(35)-Cu(39) = 1.899 and N(33)-Cu(39) = 1.892 Å. The bond angles of ligand moiety are changed upon chelation with Co(II) and Cu(II) ions. The largest changes occur for those including the two nitrogen atoms and two naphtholate

oxygens supporting their coordination to the metal centre (Tables S3 and S5). For example, the computed bond angles around Co atom are O(38)-Co(39)-O(37) = 102.899, O(38)-Co(39)-N(35) = 90.122, O(38)-Co(39)-N(33) = 116.504 and N(35)-Co(39)-N(33) 116.097 Å, which confirms the distorted tetrahedral geometry of the complex. The calculated

TABLE 7 XRD spectral data of the highest value of intensity of H_2L and its metal complexes (1-7).

Comp.	Size of particles (nm)	θ	Intensity	d-spacing (Å)	FWHM
H ₂ L	29.5601	12.6150	1564	3.5275	5.3410x10 ⁻³
Complex 1	22.4460	3.6550	171	12.0912	6.8780x10 ⁻³
Complex 2	42.8741	6.2350	500	7.0899	3.6157×10 ⁻³
Complex 3	88.8330	4.6200	190	9.5662	1.7400x10 ⁻³
Complex 4	6.3139	12.575	264	3.5378	0.0250
Complex 5	13.7870	3.8650	439	11.4308	0.0112
Complex 6	9.8393	4.1900	127	10.5500	0.0157
Complex 7	6.3814	3.96	328	11.1610	0.0243×10 ⁻³



FIGURE 7 Computational structure of the free ligand (H₂L)

angles around Cu atom O(38)-Cu(39)bond are O(37) = 112.548, O(38)-Cu(39)-N(35) = 103.022, O(37)-Cu(39)-N(33) = 101.684, N(35)-Cu(39)-N(33) = 112.213 Å,supporting the distorted tetrahedral geometry around Cu centre. The degree of distortion from perfect tetrahedral arrangement can be detected from the difference between the minimum and maximum angles around Co(II) and Cu(II) ions.^[64] The disappearance of O-H bonds alongside the appearance of new M-O bonds revealed the participation of phenolic hydroxyl groups in chelation via de-protonation process.^[65] In most cases, the M-N bond length differ than that of M-O. This can be assigned to the electronegativity difference between the nitrogen and oxygen atoms.^[66,67]

3.10 | *In-Vitro* antibacterial and antifungal assay studies

The *in-vitro* antimicrobial activities of H₂L and the synthesized complexes (1–7) were screened toward *E. coli*, *S. aureus*, *C. albicans* and *A. flavus* applying the modified Kirby–Bauer disc diffusion method.^[24] The obtained results are presented in Table 8. The free ligand under study does not have any antibacterial or antifungal activities against the tested organisms. Complexes **3–7** displayed moderate to high activities toward *E. coli*. Also, complexes **1**, **4**, **5**, **6**, and **7** visualized temperate to elevated activities against *S. aureus*. This behaviour can be illustrated on the basis of chelation theory.^[68] This theory states that a decrease in the polarizability of the metal could enhance the lipophilicity of the metal chelates. This leads up to a breakdown of the permeability of the cells, resulting in intervention with normal cell processes. So, the chelation tends to make the Schiff bases act as more powerful and potent antimicrobial agents, inhibiting the growth of bacteria more than the parent ligand.^[69] Hence, it is claiming that the chelation process of significantly influences the activity of compounds that are potent against microbial strains. All metal chelates under interest do not have any antifungal activities against the tested organisms. E. coli was selected as the backbone of Gramnegative bacteria, S. aureus was chosen to represent Grampositive bacteria, whereas. C. albicans represented the unicellular fungi. So, the selected organisms represent a broad spectrum of test organisms. The obtained results prove the effectiveness of most investigated complexes as broad spectrum antimicrobial agents.

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3.11 | Antitumor activity studies

New anticancer agents are desperately needed to ameliorate the outcome for the great number of patients who relapse after receiving the presently available cancer therapies.^[70] The anticancer activity of H₂L and its metal chelates (1–7) was detected *in vitro* against human liver Carcinoma cell line (HEPG2). We chose the particular cell line of liver

TABLE 8 Antimicrobial activities of H_2L and its metal complexes (1-7).

		Inhibition zone diameter (mm / mg sample) ^a					
Sample		E. coli (G)	S. aureus (G ⁺)	A. flavus (Fungus)	C. albicans (Fungus)		
	Control: DMSO	0.0	0.0	0.0	0.0		
Standard	Ampicillin Antibacterial	22.0	18.0				
	agent Norfloxacin Antibacterial	30.0	31.0				
	agent Amphotericin B Antifungal agent			17.0	19.0		
	H ₂ L	0.0	0.0	0.0	0.0		
	Complex 1	13	14	0.0	0.0		
	Complex 2	0.0	0.0	0.0	0.0		
	Complex 3	10	0.0	0.0	0.0		
	Complex 4	10	13	0.0	0.0		
	Complex 5	10	11	0.0	0.0		
	Complex 6	13	13	0.0	0.0		
	Complex 7	12	13	0.0	0.0		

^aEach experiment is conducted at least three times.

Carcinoma because liver Carcinoma is the most common disease among other different types of Carcinomas in our country. Doxorubicin (Dox) was applied as a reference drug $(IC_{50} = 4.73)$ and untreated cells were used as a control. Each data point is determined as the average of three independent experiments and expressed as $M \pm SD$. Growth inhibition of 50% (IC₅₀) is determined as the compound concentrations, which caused a 50% reduction in cell proliferation during the sample incubation.^[71] The mean IC_{50} is the concentration of compound that minifies cell growth by 50% under the experimental conditions. From the obtained results (Figure 8). it is clear that the growth inhibition of the tested cells strongly affected by type of metal ion and increases in the order; $H_2L < Cu(II)$ complexes < Mn(II) complex < Co(II) complex < Ni(II) complex. This reveals that the type of metal ion play an important role in anticancer activity.^[72] According to Shier,^[73] the compounds exhibiting IC_{50} value below 5.00 µg/ml are considered strong anticancer agents, compounds display IC₅₀ value between 5.00-10.00 µg/ml are moderate while those within 10.00-25.00 µg/ml range are weak anticancer agents. H₂L showed an inhibition of cell viability and gave IC50 value 11.80 µg/ml (weak antitumor



FIGURE 8 *In-vitro* cytotoxicity of H₂L and complexes 1–7 against human liver Carcinoma cell line (HEPG2)

activity). Complexes **4**, **6** and **7** displayed a moderate activity and gave IC₅₀ values of 5.56, 7.71 and 5.67 μ g/ml, respectively. Complexes **1**, **2**, **3**, and **5** showed a strong anticancer activity against human liver Carcinoma cell line (HEPG2) and gave IC₅₀ values of 4.65, 3.97, 3.30 and 4.84 μ g/ml, respectively.

4 | CONCLUSION

Seven new nano-sized Mn(II), Co(II), Ni(II) and Cu(II) complexes with N_1 . N₆-bis((2-hydroxynaphthalin-1-yl)methinyl)) adipohydrazone (H₂L) were synthesized. The complexes were characterized using spectral (FT-IR, ¹H NMR, MS, UV-Vis, ESR), elemental and thermal analyses. The XRD studies asserted the nano-sized nature of the complexes. H₂L coordinated as dibasic tetra-dentate ligand towards Mn(II), Co(II), Ni(II) and Cu(II) ions. Mn(II) and Ni(II) complexes have octahedral geometries while Co(II) and Cu(II) complexes have distorted tetrahedral configuration. Molecular modelling was performed by the molecular mechanic calculations applying the hyper chem. 8.03 molecular modelling programme to display the most stable structural formulas. The most probable mechanism, E^* , H^* , A, S^* , and G^* values were determined from TGA curves applying Coats-Redfern and Horowitz-Metzger methods. Mn(II) complex, Co(II) complex, Ni(II) complex, and Cu(II) complex (5) exhibited a strong anticancer activity with IC_{50} of 4.65, 3.97, 3.30 and 4.84 µg/ml, Cu(II) complexes 4, 6 and 7 displayed a moderate activity with IC₅₀ of 5.56, 7.71 and 5.67 µg/ml, while H₂L showed a weak inhibition of cell viability with IC₅₀ of 11.80 µg/ml, compared with IC₅₀ value of 4.73 µg/ml for the doxorubicin. The resulting data confirm great viability of the investigated compounds as new promising anticancer agents.

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