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FULL PAPER

Novel macrocyclic Schiff base and its complexes having N_2O_2 group of donor atoms: Synthesis, characterization and anticancer screening

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Novel Schiff base [*N*′,*N*′″-(((ethane-1,2-diylbis(oxy))bis(2,1-phenylene))bis(methanylylidene))di(benzohydrazide)] was formed by the condensation reaction of benzohydrazide with 2,2′-(ethane-1,2-diylbis(oxy))dibenzaldehyde. Its reaction with various metal ions was studied and the structures of the new products were characterized using common analytical and spectroscopic methods. All the metal complexes have pronounced anticancer activities. The antimicrobial activities against Gram-negative and Gram-positive bacteria were investigated.

KEYWORDS

anticancer screening, antimicrobial activities, complexes, kinetic, metal ions, novel Schiff's base, spectroscopic analysis, thermal analysis

1 | INTRODUCTION

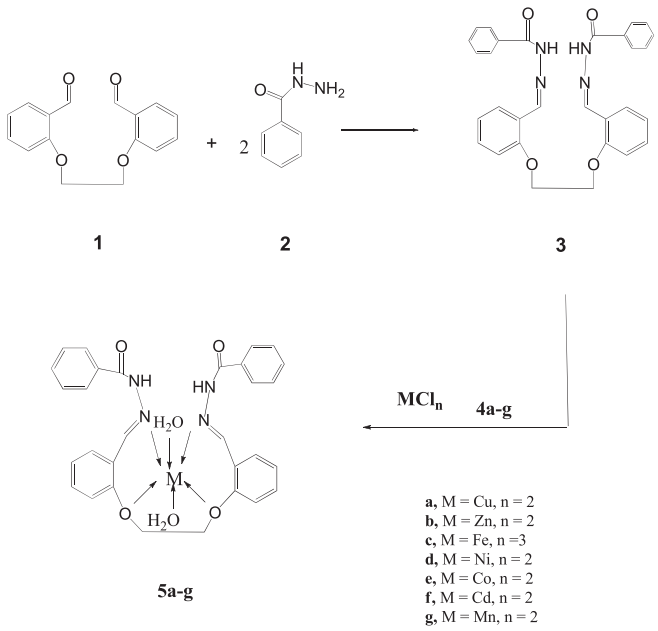
The physicochemical and pharmacological properties of heterocyclic compounds such as benzimidazoles are improved upon reaction with transition metal chlorides to give complexes.[1–4] Positively charged metal centre combined with heteroaromatic periphery forms well-defined geometries, which facilitate the interaction with biomolecules and transport across membranes in biological systems.[5,6] Thiosemicarbazone heterocyclic compounds and their metal complexes have attracted considerable attention due to their coordination chemistry and broad range of pharmacological properties.[7,8] Hydrazones are characterized by the presence of azomethine group (—CH═N—); they are good polydentate chelating agents that can form a variety of complexes with various transition metals and inner transition metals.[9–17] Metal complexes containing improved organic ligands are widely used in cancer chemotherapy. The great success in the clinical treatment of human malignancies has stimulated research in the area of inorganic antitumor agents, the application of which can be hampered by severe toxicity and development of resistance during therapy.[6,18,19] To avoid these disadvantages, current strategies for the development of novel metallo drugs have focused on the use of transition metal complexes.[11,20] Various coordination compounds have been synthesized and the effects of metal, ligand and substituent on biological and anticancer activities have been investigated.[21–23] However, side effects, toxicity, cancer specificity and especially acquired resistance are still significant problems.

The main goal of the research reported here was to prepare and characterize a biologically active heterocyclic ligand. This was reacted with metal chlorides to yield complexes having anticancer and other biological activities.

2 | EXPERIMENTAL

2.1 | Materials and reagents

All chemicals used in this study were of analytical reagent grade and of the highest purity available. They included Cu(II) chloride (Sigma), Co(II) chloride hexahydrate and



SCHEME 1 Synthesis of metal complexes **5**

Ni(II) chloride hexahydrate (BDH), ferric chloride hexahydrate (Prolabo), zinc chloride (Ubichem) and Cd(II) chloride (Aldrich). The other materials included 2,2′-(ethane-1,2-diylbis(oxy))dibenzaldehyde, salicylaldehyde, 1,2-dibromoethane (Sigma) and benzohydrazide (Aldrich). Organic solvents used included absolute ethyl alcohol and dimethylformamide (DMF). These solvents were spectroscopically pure from BDH. Double-distilled water collected using all-glass equipment was used in all preparations.

2.2 | Instrumentation

Elemental microanalyses of the separated solid chelates for C, H, N and Cl were performed at the Microanalytical Centre, Cairo University, using a CHNS-932 (LECO) Vario elemental analyser. The analyses were repeated twice to check the accuracy of the data. The molar conductance of solid chelates in DMF was measured using a Jenway 4010 conductivity meter. Fourier transform infrared (FT-IR) spectra were recorded with a PerkinElmer FT-IR type 1650 spectrophotometer in the wavenumber region 400–4000 cm^{-1}. The spectra were recorded as KBr pellets. Solid reflectance spectra were measured with a Shimadzu 3101pc spectrophotometer. The molar magnetic susceptibility was measured with powdered samples using the Faraday method. Diamagnetic corrections were made using Pascal's constant and $Hg[Co(SCN)_4]$ was used as a calibrant. Mass spectra were recorded with the EI technique at 70 eV using an MS-5988 GS-MS Hewlett-Packard instrument at the Microanalytical Centre, Cairo University. ^{1}H NMR spectra were recorded using a 300 MHz Varian-Oxford Mercury. The solvent used was deuterated dimethylsulfoxide (DMSO-d_6) and the spectra extended from 0 to 15 ppm. Thermal analyses (thermogravimetry (TG) and differential thermogravimetry (DTG)) were carried out in a dynamic nitrogen atmosphere (20 ml min^{-1}) with a heating rate of 10 °C min^{-1} using a DTG-60 H Shimadzu simultaneous DTA/TG apparatus.

2.3 | Synthesis of metal complexes 5

The metal complexes **5** were prepared by the addition of a hot solution (60 °C) of the appropriate metal chloride **4** in absolute ethanol (15 ml) to a hot solution (60 °C) of the organic ligand **3** (0.3 g) in ethanol and DMF (15 ml). The resulting mixture was heated with stirring to evaporate all the solvents to afford a precipitate. The precipitate was dried and weighed to calculate the yield. All the above steps were repeated for all the selected transition metal complexes.

2.4 | Biological activity

Testing was done using the diffusion agar technique. Spore suspension (0.5 ml, 10^6–10^7 spores ml^{-1}) of each of the investigated organisms was added to a sterile agar medium just before solidification, then poured into sterile Petri dishes (9 cm in diameter) and left to solidify. Using a sterile cork borer (6 mm in diameter), three holes (wells) were made into each dish, and then 0.1 ml of the test compound dissolved in DMF (100 mg ml^{-1}) was poured into these holes. The dishes were incubated at 37 °C for 48 h where a clear or inhibition zone was detected around each hole. DMF (0.1 ml) was used as a control under the same conditions. By subtracting the diameter of the inhibition zone resulting from DMF from that obtained from each metal complex or the free Schiff base, antibacterial activities were calculated as a mean of three replicates. MIC_{50} was determined, defined as the lowest compound concentration that inhibits growth by 50%.

2.5 | Pharmacology materials and methods

MCF-7 breast cancer cell line was obtained from the National Cancer Institute (Cairo, Egypt). MCF-7 cells were grown in RPMI-1640. Media were supplemented with 10% heat-inactivated foetal bovine serum, 50 U ml^{-1} penicillin and 50 g ml^{-1} streptomycin and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were maintained as 'monolayer culture' by serial sub-culturing. Cytotoxicity was determined using the sulforhodamine B (SRB) method as previously described.[21] Exponentially growing cells were collected using 0.25% trypsin–EDTA and seeded in 96-well plates at 1000–2000 cells per well in RPMI-1640 supplemented medium. After 24 h, cells were incubated for 72 h with various concentrations of the test compounds as well as doxorubicin as reference drug. Following 72 h of treatment, the cells were fixed with 10% trichloroacetic acid for 1 h at 4 °C. Wells were stained for 10 min at room temperature with 0.4% SRB stain dissolved in 1% acetic acid. The plates were air-dried for 24 h and the dye was solubilized with Tris–HCl for 5 min on a shaker at 1600 rpm. The optical density of each well was measured spectrophotometrically at 564 nm with an ELISA microplate

reader (ChroMate-4300, FL, USA). The IC_{50} values were calculated according to the equation for Boltzmann sigmoidal concentration–response curve using nonlinear regression fitting models (GraphPad Prism, Version 5).

3 | RESULTS AND DISCUSSION

3.1 | Characterization of metal complexes

Ligand **3** was formed by the condensation reaction of dibenzaldehyde derivative **1** with benzohydrazide (**2**)[24] (Scheme 1). The reactions of ligand **3** with metal ions **4** in equal molar ratio afforded metal complexes **5** (Scheme 1); their elemental analyses, yields and melting points are presented in Table 1. The ^{1}H NMR spectra of metal complexes **5** show the absence of a signal of NH group in the free ligand at 8.71 ppm due to the chelating process with metal. The characteristic signals of the ^{13}C NMR spectrum for complex **5b** as an example show that metal ions influence the electronic charge distribution around particular carbons which appear at 72.2 ppm (CH_2) and 150.4 ppm (CH═), and slightly change that of other carbons at 113.2 (CH), 117 (CH), 121.3 (CH), 125 (CH), 127 (CH), 134.7 (CH), 135.1 (CH), 157 (C─O) and 162 ppm (C═O). This means that the electron densities around the referred carbons are affected by the ligand interaction with metal cations to form their metal complexes.[25–28]

3.2 | Molar conductance measurements

Conductivity measurement of metal chelates in non-aqueous solutions has been used in structural studies within the limits of their solubility. This method gives the degree of ionization of compounds **5**, the molar conductivity increasing with increasing amount of ions that a complex liberates in solution. The molar conductivities of 10^{-3} molar solutions of the metal chelates at 25 ± 2 °C are given in Table 1. From the molar conductance values (108–150 Ω^{-1} mol^{-1} cm^2) of Co(II), Ni(II), Cu(II), Mn(II), Cd(II) and Zn(II) complexes, it is concluded that these complexes are 1:2 electrolytes. On the other hand, the Fe(III) complex has a molar conductance value of 250 Ω^{-1} mol^{-1} cm^2, indicating its ionic nature and it is considered as a 1:3 electrolyte.[29–31]

3.3 | FT-IR spectra and mode of bonding

The FT-IR spectra of all complexes **5** show absorption bands ν(C═N) at 1586–1656 cm^{-1} which are shifted by 50–52 cm^{-1} to lower energy regions compared to compound **3**.[32–35] This is due to the coordination of azomethine nitrogen to metal ion.[36,37] Also, a broad $\nu(H_2O)$ band of **5** is found at 3260 cm^{-1}.[38,39] The stretching band of ν(C─O─C) is observed at 1226 cm^{-1} in the spectrum of **3** which is shifted to higher wavenumbers (1253–1261 cm^{-1}) in the spectra of **5** due to the participation of the oxygen atom in chelation. New absorption bands appear in the spectra of complexes corresponding to stretching vibrations ν(M─O) and ν(M─N) in the regions 505–609 and 435–484 cm^{-1}, respectively.[40]

3.4 | Electronic spectral and magnetic susceptibility studies (Table 2)

Electronic spectra of complexes **5b** and **5f** show bands at 26 000 and 24 500 cm^{-1}, respectively, which may be due to ligand–metal charge transfer. Molar conductivities of **5b** and **5f** are over 100 Ω^{-1} cm^2 mol^{-1} as expected for 1:2 electrolytes of Zn(II) and Cd(II) complexes.[41–44]

3.5 | Thermal analyses (TG and DTG) and thermodynamic calculations

TG analysis of the ligand shows two successive steps of decomposition. The first mass loss of 16% (15.41%) in the temperature range 50–250 °C may be due to the decomposition of benzene molecule. At 250–400 °C the mass loss of 34% (34.13%) may be for the decomposition of $C_8H_7N_3O_2$ molecule. In the final stage from 400 to 600 °C, the estimated mass is loss of 50% (50.30%) is due to $C_{16}H_{13}NO_2$ molecule with complete decomposition.

TABLE 1 Analytical and physical data of metal complexes **5**

Complex	Colour (% yield)	M.p. (°C)	% Found (calcd)					μ_{eff} (BM)	Λ_m (Ω^{-1} mol^{-1} cm^2)
			C	H	N	O	M		
$C_{30}H_{30}Cl_2CuN_4O_6$ [Cu(L).2H$_2$O].Cl$_2$ (**5a**)	Brown (77)	220	53.22 (53.11)	4.47 4.25)	8.28 (8.13)	14.18 (13.78)	9.39	1.5	108
$C_{30}H_{30}Cl_2ZnN_4O_6$ [Zn(L).2H$_2$O].Cl$_2$ (**5b**)	Yellowish white (80)	215	53.08 (52.89)	4.45 (4.55)	8.25 (8.12)	14.14 (13.98)	9.63	Dia.	120
$C_{30}H_{30}FeCl_2N_4O_6$ [Fe(L).2H$_2$O].Cl$_3$ (**5c**)	Brown (75)	284	47.37 (47.21)	3.98 (3.54)	7.37 (7.26)	12.62 (12.52)	14.68	3.8	250
$C_{30}H_{30}Cl_2NiN_4O_6$ [Ni(L).2H$_2$O].Cl$_2$ (**5d**)	Greenish yellow (88)	255	53.60 (53.42)	4.50 (4.23)	8.34 (8.22)	14.28 (14.16)	8.73	2.4	118
$C_{30}H_{30}Cl_2CoN_4O_6$ [Co(L).2H$_2$O].Cl$_2$ (**5e**)	Dark red (78)	280	53.59 (53.58)	4.50 (4.26)	8.33 (7.89)	14.28 (13.87)	8.76	3.06	130
$C_{30}H_{30}Cl_2CdN_4O_6$ [Cd(L).2H$_2$O].Cl$_2$ (**5f**)	Pale yellow (80)	265	49.64 (49.32)	4.17 (4.56)	7.72 (7.48)	13.22 (13.54)	15.49	Dia.	125
$C_{30}H_{30}Cl_2MnN_4O_6$ [Mn(L).2H$_2$O].Cl$_2$ (**5 g**)	Pale yellow (86)	250	53.91 (53.28)	4.52 (4.31)	8.38 (8.26)	14.36 (14.12)	8.22	3.69	150

TABLE 2 Electronic spectral data and magnetic susceptibility of complexes **5a**, **5c–e** and **5 g**

Compound	Bands (cm^{-1})	Transitions	Magnetic moment (BM)	Geometry
5a	15 331 16 588 21 188	2B1g → 2B2g 2B1g → 2Eg 2B1g → 2A1g	1.5	Octahedral[45,46]
5c	22 754 16 550–18 321 13 822–17 436	6A1g → T2 g(G) 6A1g → 5T1g	3.8	Octahedral[42]
5d	15 785 17 406 19 588	3A2g → 3T2g 3A2g → 3T1g (F) 3A2g → 3T1g (P)	2.4	Octahedral[43,44]
5e	17 452 19 569 24 459	4T1g(F) → 4 T2 g(F) 4T1g(F) → 4A2g(F) 4T1g(F) → 4 T2 g(P)	3.06	Octahedral[43,44]
5 g	15 810 17 513	4T1g → 6A1g 4 T2 g(G) → 6A1g 4T1g(D) → 6A1g	3.69	Octahedral[41]

The TG analysis of complex **5a** as an example shows decomposition at 44–800 °C (Table 3). At 44–193 °C the complex loses 2HCl, O_2 and NO molecules with a mass loss of 20% (20.08%) as a first step. The second step corresponds to $2H_2O$, N_2 and $4CH_4$ molecules with a mass loss of 18% (18.9%) within the range 196–364 °C. The third step of decomposition occurs at 366–457 °C for loss of C_2H_5 molecule with a mass loss of 4% (4.28%). The final stage within the range 457–715 °C shows loss of $C_{24}H_3N$ with a mass loss of 45% (45.79%) leaving CuO as a residue.

Three decomposition steps appear in the thermal analysis of complex **5c** as an example of trivalant complex (Table 3). The first one corresponds to $2H_2O$ and $1/2O_2$ molecules at 24–194 °C with mass loss of 7% (6.84%). The second (198–407 °C) corresponds to the loss of some of organic ligand, 3HCl and CH_4 molecules with mass loss of 16% (16.31%). The final stage at 407–699 °C corresponds to the loss of $C_{29}H_{19}N_4$ molecule with mass loss of 56% (56.18%) and Fe_2O_3 as a residue.

TABLE 3 Thermal analysis (TG and DTG) results for complexes **5**

Compound	TG range (°C)	DTG_{max} (°C)	*n**	Mass loss, calcd (estim.) (%)	Total mass loss, calcd (estim.) (%)	Assignment	Residue
$[Cu(L).2H_2O]Cl_2$ (**5a**)	44–193	84	1	20.08 (20)	88.37 (88)	Loss of $2HCl,O_2$ and NO	CuO
	196–364	301	1	18.9 (18)		Loss of $2H_2O$, N_2 and $4CH_4$	11.7 (11)
	366–457	406	1	4.28 (4)		Loss of C_2H_5	
	457–715	561	1	45.79 (45)		Loss of $C_{24}H_3N$	
$[Zn(L).2H_2O]Cl_2$ (**5b**)	37–108	75	1	5.30 (5)	87.58 (87)	Loss of $2(H_2O)$	ZnO
						Loss of 2(HCl) and $1/2O_2$	12.42 (12)
	112–229	170	1	12.97 (13)		Loss of $2(C_2H_5)$ and NO	
	231–429	307	1	13.56 (14)		Loss of$C_{26}H_{14}N_3O$	
	431–997	615	1	55.75 (55)			
$[Fe(L).2H_2O]Cl_3$ (**5c**)	24–194	138	1	6.84(7)	79.33(79)	Loss of $2(H_2O)$ and $1/2O_2$	Fe_2O_3
	198–407	308	1	16.31(16)		Loss of 3(HCl) and CH_4	20.67 (21)
	407–699	499	1	56.18 (56)		Loss of $C_{29}H_{19}N_4$	
$[Ni(L).2H_2O]Cl_2$ (**5d**)	23–97	56	1	5.35 (6)	89.71 (89)	Loss of $2(H_2O)$	NiO
	98–354	286	1	20.23 (20)		Loss of 2(HCl) and 2(NO)	11.11 (11)
	355–452	401	1	13.69 (13)		Loss of C_2H_5, N_2O and CH_4	
	453–654	518	1	50.44 (50)			
$[Co(L).2H_2O]Cl_2$ (**5e**)	26–115	74	1	4.76 (5)	88. 86 (88)	Loss of $2(H_2O)$	CoO
						Loss of HCl	11.14 (12)
	117–204	155	1	5.35 (5)		Loss of HCl and NO	
	206–393	294	1	14.77 (14)		Loss of $C_{30}H_{24}N_2O$	
	395–710	517	1	63.98 (64)			
$[Cd(L).2H_2O]Cl_2$ (**5f**)	23–238	152	1	4. 95 (5)	82.94 (82)	Loss of $2(H_2O)$	CdO
						Loss of 2(HCl), 2(NO) and $2(CH_4)$	17.06 (18)
	240–461	341	1	23. 17 (23)		Loss of $C_{28}H_{16}N_2O$	
	461–792	605	1	54.82 (54)			
$[Mn(L).2H_2O]Cl_2$ (**5 g**)	24–204	79	1	5.38 (5)	89. 8 (89)	Loss of $C_2H_4N_4S_2$	MnO
						Loss of 2(HCl) and $1/2O_2$	$C_{12}H_8$
	204–386	311	1	17.81 (18)		Loss of $C_4H_{10}O_2N_2$	
	387–658	514	1	66.61 (66)			10.2 (11)

3.6 | Calculation of activation thermodynamic parameters

The activation energies of decomposition of the new compounds are found to be in the range 80.01–964.90 kJ mol^{-1}, these high values of the activation energies reflecting the thermal stability of the complexes (Table 4). On the other hand, the entropy of activation has a negative value for all the complexes, which indicates that the decomposition reactions proceed with a lower rate than the normal ones.

3.7 | Biological activity

The Schiff base **3** and its metal complexes **5** were tested in terms of antibacterial activity using the diffusion agar method.[45–52] The reference compound for antibacterial activities was streptomycin and more than one test organism was used. The antibacterial activity data for compounds **3** and **5** have a wide degree of variation (Table 5).

The bis-Schiff base ligand **3** has more sensitivity towards Gram-positive than Gram-negative bacteria and has high MIC_{50} (>100 mg ml^{-1}) for both types of bacteria. It is found that Cu(II) compound **5a** and Ni(II) compound **5d** have the highest inhibition zones and high MIC_{50} (>100 mg ml^{-1}) against Gram-negative bacteria (*E. coli* and *P. vulgaris*). On the other hand, complex **5e** has moderate inhibitory activity (20 and 22 mm) for *E. coli* and *P. vulgaris*, respectively, but lowest MIC_{50} (>50 mg ml^{-1}) for both microorganisms. Mn (II) compound 5 g poorly inhibits *E. coli* and *P. vulgaris* against two Gram-negative bacteria; whereas forms **3**, **5b** and **5f** are found to possess slightly inhibited significant activity. Fe (III) compound **5c** shows comparatively weak inhibition against Gram-positive bacterium *S. pyogenes* at MIC50 (>100 mg ml^{-1}).

TABLE 5 Antibacterial activity data for compounds **3** and **5**

	Inhibition zone (mm mg^{-1} sample)/MIC_{50} (mg ml^{-1})			
	Gram negative		Gram positive	
Sample	*Escherichia coli*	Proteus vulgaris	Bacillus subtilis	Streptococcus pyogenes
3	25	26	25	25
5a	30/>100	31/>100	31/>100	34/>100
5b	25/>100	28/>100	29/>100	27/>100
5c	18/>100	19/>100	20/>100	22/>100
5d	32/>100	30/>100	31/>100	34/>100
5e	20/>50	22/>50	23/>25	25/>50
5f	28/>100	29/>100	27/>100	25/>100
5 g	24/>100	22/>100	23/>100	21/>100

3.8 | *In vitro* cytotoxic activity and anticancer screening studies

The results are reported in Table 6 for three separate experiments. Statistical differences were analysed according to one-way ANOVA tests wherein the differences were considered to be significant at $p < 0.05$.

Anti-proliferative activities of the new metal complexes of Co, Cu, Zn, Cd, Fe, Ni and Mn were examined in MCF-

TABLE 4 Thermodynamic data for complexes **5**[a]

Complex	Decomposition temp. (°C)	A (s^{-1})	E (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})
$[Cu(L).2H_2O]Cl_2$ (**5a**)	44–193	4.10×10^5	52.86	−107.26	46.46	128.97
	196–364	9.02×10^6	28.72	−244.18	280.82	92.96
	366–457	7.46×10^8	98.35	−82.95	97.71	104.94
	457–715	7.56×10^7	79.51	−101.98	155.12	80.01
$[Zn(L).2H_2O]Cl_2$ (**5b**)	37–108	4.68×10^6	229.16	−39.18	222.76	786. 83
	112–229	1.93×10^7	278.90	−34.61	272.08	569.73
	231–429	9.46×10^8	385.32	−34.05	378.92	117.16
	431–997	1.57×10^5	711.82	15.33	647.82	182.74
$[Fe(L).2H_2O]Cl_3$ (**5c**)	24–197	2.87×10^6	307.53	−44. 52	301.13	413. 78
	198–407	2.96×10^7	469.44	−48. 37	46.30	908.69
	407–699	6.57×10^6	134.81	−64.85	128.41	178.31
$[Ni(L).2H_2O]Cl_2$ (**5d**)	23–97	1.17×10^5	308.29	−64.83	301.89	196.91
	98–354	6.36×10^6	918.06	−136. 37	858.68	183. 56
	355–452	2.81×10^7	803.99	−90.47	797.59	101.60
	453–654	4.84×10^8	689.79	−105.67	497.36	817.96
$[Co(L).2H_2O]Cl_2$ (**5e**)	26–115	$6. 81 \times 10^6$	608.29	−131. 40	601.76	409. 12
	117–204	1.54×10^7	485.92	−76. 55	479.52	109.40
	206–393	1.35×10^8	390.11	−32.40	383.71	134.39
	395–710	1.91×10^{11}	127.61	−36.90	636.66	347.47
$[Cd(L).2H_2O]Cl_2$ (**5f**)	23–238	1.91×10^6	53.80	39.68	537.97	537.67
	240–461	9.63×10^7	96.55	72.35	965.45	964.90
	461–792	6.36×10^8	144.60	77.71	144.60	144.60
$[Mn(L).2H_2O]Cl_2$ (**5 g**)	24–204	1.68×10^7	110.48	195.53	104.08	890.39
	204–386	4.09×10^8	246.79	21.84	240.39	723.64
	387–658	7.22×10^9	523.47	35.71	517.07	242.307

[a]*E*, activation energy; *H*, enthalpy; *S*, entropy; *G*, Gibbs free energy.

TABLE 6 *In vitro* anti-proliferative activities of the newly prepared derivatives against various cell lines

Compound[a]	IC_{50} (μg $μl^{-1}$)[b]		
	MCF-7	HepG2	HCT
5e	14.8 ± 0.02	10.6 ± 0.01	8.2 ± 0.012
5a	10.3 ± 0.01	4.7 ± 0.007	3.7 ± 0.005
5b	4.8 ± 0.007	3.1 ± 0.004	11.2 ± 0.016
5f	4.4 ± 0.006	3.3 ± 0.004	5.9 ± 0.008
5c	11.9 ± 0.017	9.4 ± 0.014	8.6 ± 0.013
5d	12.2 ± 0.018	5.3 ± 0.007	3.9 ± 0.005
5 g	4.4 ± 0.006	10.5 ± 0.015	5.7 ± 0.008
DOX	4.6 ± 0.008	ND	ND
DOX	ND	1.2 ± 0.002	ND
DOX	ND	ND	4.69 ± 0.008

[a]DOX, doxorubicin (standard drug)

[b]IC_{50} values are mean ± SD of three separate experiments. ND, not detected.

7 (breast cancer) cell line (Figure 1), HepG2 (perpetual) cell line and HCT (colon cancer) cell line using doxorubicin colorimetric assay as described previously.[53]

Doxorubicin was used as a reference cytotoxic compound for the MCF-7, HepG2 and HCT cell lines. The growth inhibitory concentration (IC_{50}) values, which refer to the concentration of compound required to produce a 50% inhibition of cell growth after 72 h of incubation compared to untreated controls, are summarized in Table 6. The complexes for which cell growth was inhibited by more than 50% are assigned as active. Almost all heterocyclic transition metal(II) complexes did not show cytotoxic activity and did not enter the secondary screening. Transition metal complexes with Zn, Cd and Mn show high specificity and are more potent for MCF-7 cell line compared with doxorubicin analogue with IC_{50} = 4.6 μM. This is due to chelation of Schiff bases of our heterocyclic compounds containing an azomethine group (—CH═N—) bond.

Also, the heterocyclic transition metal complexes were screened against HepG2 cells (Figure 2). The results show moderate activity for the Zn metal complex at IC_{50} = 3.1 μM compared with doxorubicin analogue with IC_{50} = 1.2 μM. This means that none of the complexes possesses the ability to inhibit the growth of cancer cell lines at 20 μM.

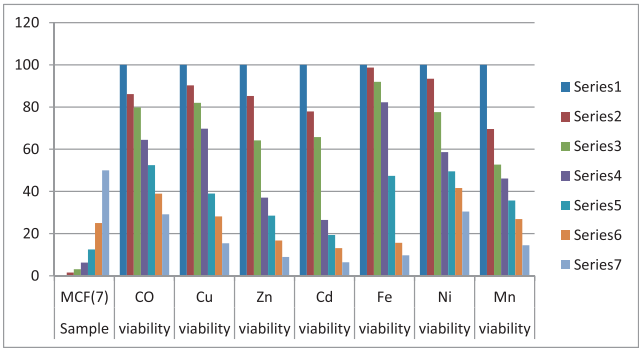


FIGURE 1 The growth inhibitory IC_{50} values [μM] of metal (II) complexes **5** at the concentration of 20 μM for MCF-7 (breast cancer cells)

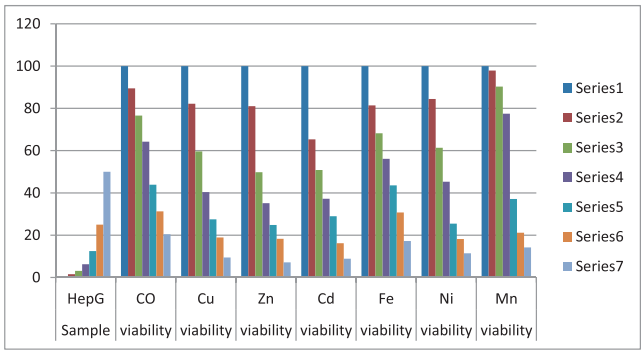


FIGURE 2 The growth inhibitory IC_{50} values [μM] of metal (II) complexes **5** at the concentration of 20 μM for HEPG-2 (liver cancer cells)

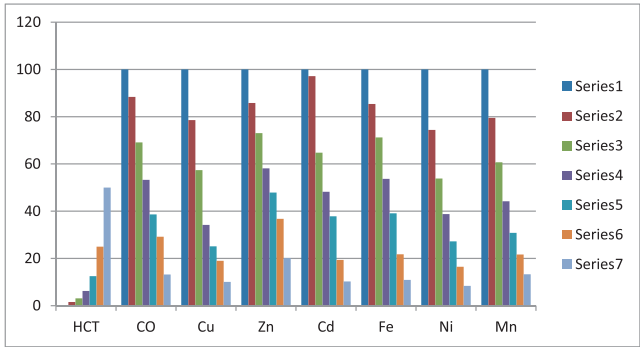


FIGURE 3 The growth inhibition IC_{50} values [μM] of metal (II) complexes **5** at the concentration of 20 μM for HCT (colony cancer cells)

Finally the transition metal(II) complexes were tested against HCT cell line (Figure 3). The Cu(II) complex shows higher activity at IC_{50} = 3.7 μM compared with doxorubicin analogue at IC_{50} = 4.69 μM, which indicates that the Cu(II) complex exhibits cytotoxic activity against HCT cell line.

4 | CONCLUSIONS

In this study a novel Schiff base and novel synthesized heterocyclic transition metal complexes were developed via a delivery system emulated by self-assembly of doxorubicin. Their biological, *in vitro* cytotoxic and anticancer activities were investigated. The Cu(II) complex increased the accumulation of doxorubicin in tumour cells (HCT). The Cd, Zn and Mn complexes increased the efficacy in breast cancer cells (MCF-7). The metal complexes have greater antimicrobial effect than the free ligand.

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