

Synthesis, spectral characterization, thermal, anticancer and antimicrobial studies of bidentate azo dye metal complexes

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Abstract As a part of systematic investigation of biologically active compound, m-phenylene diamine, new azo dye ligand was synthesized by diazotization of *m*-phenylene diamine and coupled with coupling compound, pmethoxy benzaldehyde. A new series of Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes derived from this azo dye ligand (L) were synthesized. The structures of the ligand and metal complexes were confirmed by elemental analysis, spectroscopic studies (IR, UV–Vis, ¹H NMR, mass spectrometry, electronic spectra, magnetic susceptibility and ESR), conductivity measurements, thermogravimetric analyses (TG-DTG) and X-ray powder diffraction. The molar conductance measurements of the complexes in DMF determine electrolytic nature of the complexes. On the basis of elemental and thermal analyses, magnetic moment, electronic and ESR spectral studies, an octahedral geometry was assigned for metal complexes. XRD data reflect that azo dye ligand and its Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes are amorphous while Cd(II) complex is crystalline. Also, the newly synthesized azo dye ligand, in comparison with metal complexes, is screened for its antimicrobial and anticancer activity against breast cancer cell line (MCF7). The results showed that Mn(II), Ni(II) and Zn(II) metal complexes have higher anti-breast cancer activity than free ligand.

Keywords p-Methoxybenzaldehyde \cdot Spectroscopy \cdot Kinetics studies \cdot Antimicrobial activity \cdot Anticancer activity

Introduction

In the last few years, chemists have taken a growing interest in the synthesis and physico-chemical studies of first row transition metal complexes with a number of azo dye ligands. Metal complexes of azo dye ligand have played a central role in the development of coordination chemistry [1]. Azo compounds are a very important class of chemical compounds receiving attention in scientific research. They are highly colored and they were studied widely because of their excellent thermal and optical properties in applications such as optical recording medium, toner, ink-jet printing and oil-soluble lightfast dyes [2]. Azo compounds are known to be involved in a number of biological reactions, such as inhibition of DNA, RNA, and protein synthesis, nitrogen fixation, and carcinogenesis [3]. Azo compounds were the subject of many spectral studies for purpose of identification or structure elucidation, as they are capable of forming metal chelates with transition metal ions [4]. Metal-azo complexes are currently being involved as recording layers in a number of high-density optical storage systems such as blue-ray discs (BDs) and high-density DVDs (HD-DVDs). For this purpose, new azo dye ligands derived from (3,3'-((1Z,1Z')pyridine-2,6-diylbis(diazene-2,1-diyl))bis(4-methoxybenzaldehyde)) was prepared using the recommended methods. The composition and stability of the complexes of the

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newly synthesized azo dye ligand with Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) were investigated using elemental analyses, IR, ¹H NMR, ESR, UV–Vis, XRD, conductivity, mass spectra and magnetic susceptibility measurements at room temperature, thermal analyses. The azo dye ligand and its metal complexes were screened for their antimicrobial and anticancer activity.

Experimental

Materials and reagents

All chemicals used in this study were of pure grade and of highest purity available and used without further purification. The chemicals used included *m*-phenylene diamine (Sigma), *p*-methoxy-benzaldehyde (Aldrich), CrCl₃·6H₂O (Sigma), MnCl₂·2H₂O (Sigma), NiCl₂·6H₂O (BDH), FeCl₃·6H₂O (Prolabo), CoCl₂·6H₂O (Aldrich), CuCl₂·2H₂O (Merck), ZnCl₂ (Strem Chemicals) and CdCl₂ (Aldrich). Organic solvents were spectroscopic pure from BDH included ethanol and dimethyl formamide. Hydrochloric acid, sodium nitrite and sodium acetate (A.R.) were used.

Human tumor cell line (breast cell line MCF7) was obtained frozen in liquid nitrogen (-180 °C) from the American Type Culture Collection and was maintained in the National Cancer Institute, Cairo, Egypt, by serial subculturing.

Solutions

Stock solutions of metal complexes of 1×10^{-3} M were prepared by dissolving the accurately weighed amount of the complexes in DMF for measuring conductivity. Solutions of the azo dye ligand and metal complexes ranging from 1×10^{-4} to 1×10^{-5} M were prepared by accurate dilution of the previous prepared stock solutions to measure UV–Vis spectra.

Solutions for anticancer activity studies

A fresh stock solution of 1×10^{-3} M of azo dye ligand (0.00402 g L⁻¹) was prepared in the appropriate volume of DMF. Dimethyl sulfoxide (DMSO) used in cryop-reservation of cells and RPMI-1640 medium were supplied from Sigma. The medium was used for culturing and maintenance of the human tumor cell line. The medium was supplied in a powder form. It was prepared as follows: 10.4 g medium was weighed, mixed with 2 g sodium bicarbonate, completed to 1 L with distilled water

and shaked carefully till complete dissolution. The medium was then sterilized by filtration in a Millipore bacterial filter (0.22 µm). The prepared medium was kept in a refrigerator (4 °C) and checked at regular intervals for contamination. Before use, the medium was warmed at 37 °C in a water bath and supplemented with penicillin/ streptomycin and FBS. Sodium bicarbonate (Sigma Chemical Co., St. Louis, Mo, USA) was used for the preparation of RPMI-1640 medium. 0.05 % isotonictrypan blue solution (Sigma Chemical Co., St. Louis, Mo, USA) was prepared in normal saline and was used for viability counting. 10 % fetal bovine serum(FBS) (heat inactivated at 56 °C for 30 min), 100 units mL⁻¹ penicillin and 2 mg mL⁻¹ streptomycin were supplied from Sigma Chemical Co., St. Louis, Mo, USA, and were used for the supplementation of RPMI-1640 medium prior to use. 0.025 % (w/v) trypsin was used for the harvesting of cells, 1 % (v/v) acetic acid was used for dissolving the unbound SRB dye, and 0.4 % sulforhodamine-B (SRB) dissolved in 1 % acetic acid was used as a protein dye. Stock solution of trichloro acetic acid (TCA, 50 %) was prepared and stored. All of them were supplied from Sigma. Fifty microliters of the stock solution was added to 200 µl RPMI-1640 medium/well to yield a final concentration of 10 % used for protein precipitation. 100 % isopropanol and 70 % ethanol were used. Tris base 10 mM (pH 10.5) was used for SRB dye solubilization. 121.1 g of tris base was dissolved in 1000 mL of distilled water and pH was adjusted by HCl (2 M).

Measurements

Microanalyses of carbon, hydrogen and nitrogen were carried out at the Microanalytical Center, Cairo University, Egypt, using CHNS-932 (LECO) Vario Elemental Analyzer. Analyses of the metals followed the dissolution of the solid complexes in concentrated HNO₃, neutralizing the diluted aqueous solutions with ammonia and titrating the metal solutions with EDTA. ¹H NMR spectra, as a solution in DMSO-d6, were recorded on a 300 MHz Varian-Oxford Mercury at room temperature using TMS as an internal standard. Electron spin resonance spectra were also recorded on JES-FE2XG ESR spectrophotometer at Microanalytical Center, Tanta University. The X-ray powder diffraction analyses were carried out using Philips Analytical X-ray BV, diffractometer-type PW 1840. Radiation was provided by copper target (Cu anode 2000 W) high-intensity X-ray tube operated at 40 kV and 25 mA. Divergence and the receiving slits were 1 and 0.2, respectively. Mass spectra were recorded by the EI technique at 70 eV using MS-5988 GS-MS Hewlett–Packard instrument at the Microanalytical Center, National Center for Research, Egypt. FTIR spectra were recorded on a Perkin-Elmer 1650 spectrometer ($4000-400 \text{ cm}^{-1}$) in KBr pellets. The electronic spectra were recorded in DMSO at

spectrophotometrically at 564 nm with an ELIZA microplate reader (Meter tech. R 960, USA).

Synthesis of azo dye ligand



room temperature on Shimadzu UV-Visible mini-1240 spectrophotometer. The molar magnetic susceptibility was measured on powdered samples using the Faraday method. The diamagnetic corrections were made by Pascal's constant, and Hg[Co(SCN)₄] was used as a calibrant. Molar conductivities of 10^{-3} M solutions of the solid complexes in DMF were measured using Jenway 4010 conductivity meter. The thermogravimetric analyses (TG and DTG) of the solid complexes were carried using a Shimadzu TG-50H thermal analyzer in a dynamic nitrogen atmosphere (flow rate 20 mL min⁻¹) with a heating rate of 10 °C min⁻¹. The percentage mass loss was measured from the ambient temperature up to 1000 °C. Highly sintered α -Al₂O₃ was used as a reference. Diffused reflectance spectra analyses were carried out at the Microanalytical Center, Cairo University, Egypt. The antimicrobial activities were carried out at the Microanalytical Center, Cairo University, Egypt. The anticancer activity was performed at the National Cancer Institute, Cancer Biology Department, Pharmacology Department, Cairo University. The optical density (O.D.) of each well was measured In 250-mL quickfit round bottom flask, m-phenylene diamine (3.00 g, 27.78 mmol) was dissolved in ethanol (50 mL). The solution was cooled with stirring in an ice bath to 0 °C until it becomes clear, then HCl solution (4 mL) was added. Solution of sodium nitrite (3.80 g, 55.60 mmol) in water (20 mL) was added dropwise during 10 min and the reaction mixture was further stirred for 20 min in an ice bath at 0-5 °C. The solution was added dropwise to p-methoxybenzaldehyde coupling component (7.56 g, 55.60 mmol) in ethanol (30 mL) and aqueous solution of sodium acetate (4 g) as catalyst, with stirring in an ice bath for a further 1 h. The product was collected by filtration and washed with water and dried under vacuum at room temperature. The physical and analytical data of the isolated azo dye ligand are listed in Table 1. The compound has high melting point, and it is found to be air stable [5].

Synthesis of metal complexes



Table 1 Analytical and physical data of azo dye ligand and its metal complexes

Compound	Color/%yield	M.p./°C	% Found/Calcd.					$\mu_{\rm eff}/{ m BM}$	$\Lambda_{\rm m}/\Omega^{-1}{\rm mol}^{-1}~{\rm cm}^2$
(molecular formula)			С	Н	Ν	Cl	М		
L	Reddish brown	98	65.68	4.65	13.54	_	_	_	_
$(C_{22}H_{18}N_4O_4)$	(85)		(65.67)	(4.48)	(13.93)				
$[Cr(L)(H_2O)Cl]Cl_2 \cdot H_2O$	Black	>300	44.20	3.86	9.58	18.06	8.54	3.80	118
$(C_{22}H_{22}Cl_3CrN_4O_6)$	(83)		(44.26)	(3.69)	(9.39)	(17.85)	(8.72)		
$[Mn(L)(H_2O)_2]Cl_2$	Black	>300	46.90	4.21	9.77	12.42	9.35	5.42	108
(C22H22Cl2NiN4O6)	(87)		(46.81)	(3.90)	(9.93)	(12.59)	(9.75)		
[Fe(L)(H ₂ O)Cl]Cl ₂ ·H ₂ O	Black	206	43.50	4.02	9.50	18.02	9.62	5.29	99
$(C_{22}H_{22}Cl_3FeN_4O_6)$	(78)		(43.96)	(3.66)	(9.33)	(17.74)	(9.33)		
$[Co(L)(H_2O)_2]Cl_2$	Black	230	46.10	4.21	9.97	12.63	10.45	5.30	126
$(C_{22}H_{22}Cl_2CoN_4O_6)$	(90)		(46.48)	(3.88)	(9.86)	(12.51)	(10.34)		
[Ni(L)Cl ₂]	Black	>300	49.48	3.59	10.15	12.99	11.24	3.27	38
$(C_{22}H_{18}Cl_2NiN_4O_4)$	(89)		(49.65)	(3.39)	(10.53)	(13.35)	(11.04)		
$[Cu(L)(H_2O)Cl]Cl \cdot H_2O$	Black	280	46.04	4.04	10.05	12.64	11.39	1.79	71
$(C_{22}H_{22}Cl_2CuN_4O_6)$	(85)		(46.11)	(3.84)	(9.78)	(12.40)	(11.09)		
$[Zn(L)Cl_2] \cdot H_2O$	Black	225	49.50	3.05	9.98	13.33	11.86	Diam.	18
$(C_{22}H_{20}Cl_2ZnN_4O_6)$	(81)		(49.07)	(3.34)	(10.41)	(13.20)	(12.08)		
[Cd(L)Cl ₂]	Black	162	44.83	3.25	9.12	11.99	19.44	Diam.	8
$(C_{22}H_{18}Cl_2CdN_4O_4)$	(86)		(45.10)	(3.07)	(9.57)	(12.13)	(19.20)		

Azo dye ligand was dissolved in DMF (0.4 g, 9.95×10^{-4} mmol) and ethanolic metal salts with the same molar ratio of 1:1 (M: azo dye) (0.265 g Cr(III), 0.161 g Mn(II), 0.269 g Fe(III), 0.237 g Co(II), 0.237 g Ni(II), 0.134 g Cu(II), 0.136 g Zn(II) and 0.183 Cd(II)) were added to the azo dye ligand solution. The mixture was heated under reflux for 2–3 h with stirring. The precipitates were filtered off, washed with ethanol followed by diethyl ether and dried in a vacuum desiccator over anhydrous CaCl₂.

Spectrophotometric studies

The absorption spectra were recorded for 1×10^{-4} and 5×10^{-5} M solutions of the azo dye free ligand and of its metal complexes dissolved in DMF. The spectra were scanned within the wavelength range from 200 to 700 nm.

Pharmacology

Antimicrobial activity

A filter paper disk (5 mm) was transferred into 250 mL flasks containing 20 mL of working volume of tested solution (100 mg mL⁻¹). All flasks were autoclaved for 20 min at 121 °C. LB agar media surfaces were inoculated with four investigated bacteria (Gram-positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria: *Neisseria gonorrhoeae* and *Escherichia coli*) and one strain

of fungi (Candida albicans) by diffusion agar technique, then, transferred to a saturated disk with a tested solution in the center of Petri dish (agar plates). All the compounds were placed at four equidistant places at a distance of 2 cm from the center in the inoculated Petriplates. DMSO served as control. Finally, all these Petri dishes were incubated at 25 °C for 48 h where clear or inhibition zones were detected around each disk. Control flask of the experiment was designed to perform under the same condition described previously for each microorganism but with dimethyl formamide solution only and by subtracting the diameter of inhibition zone resulting with dimethyl formamide from that obtained in each case, so antibacterial activity could be calculated [6, 7]. Amikacin and ketoconazole were used as reference compounds for antibacterial and antifungal activities, respectively. All experiments were performed as triplicate, and data plotted were the mean value.

Anticancer activity

Potential cytotoxicity of the complexes was tested using the method of Skehan and Storeng [8], cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the complexes to allow attachment of cell to the wall of the plate. Different concentrations of the complexes under investigation (0, 5, 12.5, 25, 50 and 100 μ g mL⁻¹) were added to the cell monolayer triplicate wells and were prepared for each individual dose. The monolayer cells were incubated with the complexes for 48 h at 37 °C and in 5 %

CO₂ atmosphere. After 48 h, cells were fixed, washed and stained with SRB stain. Excess stain was washed with acetic acid and attached stain was recovered with tris–EDTA buffer. The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader, and the mean background absorbance was automatically subtracted and mean values of each drug concentration was calculated. The relation between surviving fraction and drug concentration is plotted to get the survival curve of breast tumor cell line for each complex.

Calculation

The percentage of cell survival was calculated as follows: Survival fraction = O.D.(treated cells)/O.D.(control cells).

The IC₅₀ values (the concentrations of the azo dye ligand or its complexes required to produce 50 % inhibition of cell growth) were calculated. The experiment was repeated three times for MCF7 cell line.

Results and discussion

Characterization of azo dye ligand

Free ligand and its metal complexes are soluble in DMF and DMSO solvents and have high and sharp melting points which indicated their pure nature. The IR spectrum of the free azo dye ligand was carried out in the range of $4000-400 \text{ cm}^{-1}$, and the most effective bands are listed in Table 2. The small band observed at 1680 cm^{-1} , sharp band at 1599 cm^{-1} and medium band at 1164 cm^{-1} can be attributed to C=O stretching [9], N=N [10] and C-O methoxy [11], respectively.

¹H NMR spectrum of free azo dye ligand (L) in DMSO- d_6 exhibited the following signals: 7.05–8.59 ppm (m, 10H, Ar–H) [12], 3.85 ppm (s, 6H, OCH₃) [4] and 9.86 ppm (s, 2H, CHO) [13]. These signals indicated that three different types of protons are present in the azo dye ligand.

The mass spectrum of the azo dye ligand displays the predominant molecular ion peak (M⁺) at m/z = 402 with complete agreement with results of elemental analysis. The base peak appeared at m/z = 135 due to $[C_8H_7O_2]^+$ ion. The other fragments give the peaks at 76, 104, 132, 135, 207, 239, 267, 282, 338 and 370 a.m.u. with various intensities (Scheme 1).

Characterization of metal complexes

Elemental analyses

The stoichiometry and formulation of the free azo dye (L) ligand (Fig. 1) and its metal complexes were confirmed

Table 2	IR spectra (4000-400 cm	n^{-1}) of azo dye ligan	d and its metal	complexes					
L	$[Cr(L)(H_2O)Cl]Cl_2\cdot H_2O$	$[Mn(L)(H_2O)_2]Cl_2$	$[Fe(L)(H_2O) \\ CI]Cl_2 \cdot H_2O$	$ \begin{bmatrix} C_0(L)(H_2O)_2 \end{bmatrix} \\ Cl_2 \\ Cl_2 \\ \end{bmatrix} $	[Ni(L) Cl ₂]	$[Cu(L)(H_2O)CI]CI \cdot H_2O$	$[Zn(L)Cl_2]\cdot H_2O$	[Cd(L) Cl ₂]	Assignment
1680s	1648m	1648w	1649m	1645w	1660s	1650s	1654m	1640s	C=O stretching
1599sh	1604m	1603m	1600m	1604m	1606w	1603sh	1602sh	1604m	N=N
1164m	1173m	1168m	1166m	1169m	1169m	1170sh	1170m	1167m	C-O methoxy
I	930s, 835s	922s, 832m	950s, 831m	925s, 835m	I	920s, 833m	I	I	H ₂ O stretch of coordinated water
1	567w	560w	570s	561s	521w	565s	523w	527w	M-0
I	520s	520w	517w	521 w	I	524w	I	I	M-O stretch of coordinated water
I	422w	420w	442w	442w	442w	426w	426w	442w	M–N
sh sharp	o, m medium, br broad, s s	small, w weak							





by their elemental analysis (Table 1). The metal/ligand ratio was found to be 1:1 in all complexes, which have been arrived by estimating the carbon, hydrogen, nitrogen, chloride and metal contents of the complexes. The elemental analyses of the ligand and its complexes reveal good agreement with the proposed structures. The ligand and its metal complexes have high melting points, and they are found to be air stable. The azo dye ligand is soluble in common organic solvents, and all the complexes are freely soluble in DMF and DMSO but insoluble in methanol, ethanol and water.

Molar conductance measurements

Molar conductivity measurements (Table 1) showed that Cr(III), Mn(II), Fe(III) and Co(II) complexes are electrolytes of the type 1:2, while Cu(II) complex is 1:1 electrolytic type (Table 1). The data showed that Ni(II), Zn(II) and Cd(II) chelates are non-electrolytes [14, 15]. These results lead to general formulae:

 $[ML(H_2O)Cl]Cl_x \cdot yH_2O \text{ where } M = Cr(III), Fe(III);$ x = 2, y = 1 and Cu(II); x = 1, y = 1. $[ML(H_2O)_2]Cl_x \text{ where } M = Mn(II), Co(II); x = 2.$ $[MLCl_2] \cdot yH_2O \text{ where } M = Ni(II), Zn(II) \text{ and } Cd(II);$ y = 0 for Ni(II), Cd(II); y = 1 for Zn(II).

IR spectral studies

The important infrared spectral bands and their tentative assignments are discussed here. The IR spectra of the free ligand (L) and its metal complexes were carried out in the range of $4000-400 \text{ cm}^{-1}$ and the most effective bands are listed in Table 2.

On comparing the IR spectra of free azo dye ligand (L) and its metal complexes, the bands at 1599 and 1164 cm⁻¹ in the ligand due to stretching v(N=N) and v(C-O methoxy) groups, respectively, were shifted on complexation and appeared at 1600–1606 and



Fig. 1 Structure of the newly synthesized azo dye ligand

1166–1173 cm⁻¹, respectively, indicating the involvement of nitrogen of azo group in chelation and coordination of oxygen of methoxy group to metal ions [9–11]. Also, aldehydic carbonyl group showed band at 1680 cm⁻¹ in the free azo dye ligand which shifted to 1640–1660 cm⁻¹ in the metal complexes. This explained as binding of the azo group (which has withdrawing nature) to the metal ions via its lone pair of electrons will lead to decrease in the electron withdrawing effect of aldehydic group and hence affect the position of aldehydic band in the metal complexes (1640–1660 cm⁻¹) [16].

In the far IR spectra of all the complexes, the non-ligand bands observed at 521–570 and 420–442 cm⁻¹ region assigned to ν (M–O) [9, 16] and ν (M–N) stretching vibrations, respectively [10, 17].

The coordinating water in the Cr(III), Mn(II), Fe(III), Co(II) and Cu(II) complexes is characterized by the appearance of v(M–O) at 517–524 cm⁻¹. Also the stretching vibrations at 831–835 and 920–950 cm⁻¹ assigned to v(M \leftarrow OH₂), supported for the participation of water in coordination [18, 19].

¹H NMR spectral studies

The ¹HNMR spectra of the azo dye ligand and its Zn(II) and Cd(II) complexes are recorded in (DMSO- d_6) solution using tetramethylsilane (TMS) as internal standard. ¹HNMR spectrum of the ligand revealed the following signals at $\delta = 3.85$ (s, 6H, OCH₃) [4], 7.05–8.59 (m, 10H, Ar–H) [12] and 9.86 ppm (s, 2H, CHO) [13], while the ¹H NMR spectra of the Zn(II) and Cd(II) complexes revealed the following signals at $\delta = 3.73-3.86$ (m, 6H, OCH₃), 7.05–7.94 (m, 10H, Ar–H) and 9.80–9.86 ppm (s, 2H, CHO). It is found that the signal observed at 3.33 ppm for Zn(II) complex is assigned to hydrated H₂O protons. The shift in the methoxy protons indicated that oxygen atom of OCH₃ groups is coordinated to the Zn(II) and Cd(II) ions.

Mass spectral studies

Mass spectral data of free azo dye ligand and its Co(II) and Cd(II) complexes were consistent with the molecular ion

fragments and support the proposed structures (Fig. 2.). Some of the fragments observed in the mass spectra of the azo dye ligand (L) and its Co(II) and Cd(II) complexes are shown in fragmentation Schemes 1-5.

UV-Vis absorption studies

UV–Vis spectra of azo dyes are generally affected by their chemical structures such as chromophores, substituted groups, number of azo groups, metal ions, pH values, solvents. [20–24]. In order to explore and compare the differences between free ligand and its metal complexes, UV–Vis spectra of the azo dye ligand, Mn(II) and Co(II) complexes in their 5.0×10^{-5} mol L⁻¹ DMF solutions and Cr(III), Fe(III), Ni(II), Cu(II), Zn(II) and Cd(II) complexes of concentration 1.0×10^{-4} mol L⁻¹ are determined. The results showed only one peak observed at 283 nm in the free azo dye ligand which assigned to the moderate energy π – π * transition of the aromatic rings. This peak showed shifting in the metal complexes as it appears at 273–280 nm [2, 25].

Electronic spectra and magnetic moment measurements

Cr(III) binary chelate with octahedral symmetry showed three spin allowed bands at 19,250, 20,110 and 21,350 cm⁻¹. These bands may be assigned to ${}^{4}A_{2g}(F) \rightarrow {}^{4}T_{2g}(F)$, ${}^{4}A_{2g}(F) \rightarrow {}^{4}T_{1g}(F)$ and ${}^{4}A_{2g}(F) \rightarrow 4T_{1g}(P)$ transitions indicating the octahedral geometry of the complex. The magnetic moment at room temperature is 3.80 B.M. which corresponds to the expected value for octahedral Cr(III) complexes [26, 27].

Mn(II) complex showed a broadband centered at 16,500 cm⁻¹ which corresponds to ${}^{6}A_{1g} \rightarrow {}^{4}T_{1g}$ (4G) transition, and its magnetic moment value is found to be 5.42 B.M. This indicates an octahedral environment around the Mn(II) ion [26].

The diffused reflectance spectrum of the Fe(III) chelate displays band at 26,080 cm⁻¹, which may be assigned to the ${}^{6}A_{1g} \rightarrow {}^{5}T_{2g}(G)$ transition in octahedral geometry of the complex [21, 24]. The two bands at 18,390 and 16,400 cm⁻¹ are the result of splitting of ${}^{6}A_{1g} \rightarrow {}^{5}T_{1g}$ transition. The observed magnetic moment value of Fe(III) complex is found to be 5.29 B.M. indicating octahedral geometry with d²sp³ hybridization in Fe(III) complex [26, 28].

Co(II) complex exhibits three bands at 8970, 13,500 and 19,760 cm⁻¹ which corresponds to ${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(F)$, ${}^{4}T_{1g}(F) \rightarrow {}^{4}A_{2g}(F)$ and ${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{1g}(P)$, respectively, and the observed magnetic moment value (5.20 B.M.) indicates that it has an octahedral environment.

The nickel(II) complex showed four bands at 10,140, 16,125, 24,344 and 29,890 cm^{-1} which are assigned to







 ${}^{4}T_{1g}(F) \rightarrow {}^{4}T_{2g}(F), {}^{4}T_{1g}(F) \rightarrow {}^{4}A_{2g}(F), {}^{4}T_{1g}(F) \rightarrow {}^{4}T_{1g}(P)$ and $L \rightarrow M$ charge transfer, respectively. The calculated magnetic moment (3.27 BM) confirms the octahedral geometry [28].

Cu(II) complex showed three bands at 12,122, 21,800 and 23,224 cm⁻¹ corresponding to ${}^{2}B_{1g} \rightarrow {}_{2}A_{1g}$, ${}^{2}B_{1g} \rightarrow {}_{2}B_{2g}$ and ${}^{2}B_{1g} \rightarrow {}^{2}E_{g}$ transitions in octahedral geometry [26, 29]. The magnetic moment of this complex





is 1.79 B.M. which fall within the range normally observed for octahedral Cu(II) complex [29].

Zn(II) and Cd(II) complexes are diamagnetic and octahedral geometry was proposed according to the proposed formulae.

ESR studies

The ESR spectrum of the Cu(II) complex was recorded in DMSO at 300 and 77 K, and its parameters are given in (Fig. 3). The observed order for Cu(II) complex (g_{\parallel} (3.22) > g_{\perp} (2.073) indicated that the complex exerts an octahedral geometry [29–31]. The observed value of G for the Cu(II) complex (G = 1.337) implies that the exchange coupling is not present and misalignment is appreciable. The trend $g_{\parallel} > g_{\perp} >$ ge (2.0023) showed that the unpaired electron is localized in the d_{x2-y2} orbital of the Cu(II) ion in complex [5, 29, 32]. The g_{iso} (2.04) value less than 2.3 indicated the covalent character of the metal ligand bond and the α^2 value (1.87) suggestive of in-plane covalency. The calculated value ($g_{\parallel}/A_{\parallel}$) 114 cm⁻¹ for the complex is

consistent with slightly distorted structure and showed poor in-plane π bonding.

Powder X-ray diffraction spectroscopy

X-ray powder diffraction pattern in the $0^{\circ} < 2\theta < 60^{\circ}$ of the free azo dye ligand and its metal complexes were carried out in order to give an insight about the lattice dynamics of these compounds. The X-ray powder diffraction obtained reflects a shadow on the fact that each solid represents a definite compound of a definite structure which is not contaminated with starting materials. Such facts suggest that the L and its Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) complexes are amorphous while Cd(II) complex is crystalline [30].

The average crystallite size (ξ) can be calculated from the XRD pattern according to Debye–Scherrer equation [33, 34]

$$\xi = \frac{K\lambda}{\beta_{1/2}\cos\theta}.\tag{1}$$



The equation uses the reference peak width at angle (θ) , where λ is wavelength of X-ray radiation (1.541874 Å), K is constant taken as 0.95 for organic compounds [21] and $\beta_{1/2}$ is the width at half maximum of the reference diffraction peak measured in radians. The dislocation density, δ , is the number of dislocation lines per unit area of the crystal. The value of δ is related to the average particle diameter (ξ) by the relation [35, 36]:

$$\delta = \frac{1}{\xi^2}.$$
(2)

The value of ξ is calculated and found to be 0.146 nm for Cd(III) complex. The value of δ is 46.76 nm⁻² for Cd(II) complex.

Thermal analysis studies

The TG curves for the azo dye ligand and its metal complexes showed the temperature intervals and the percentage of loss of masses (Fig. 4). Steps of decomposition of azo dye ligand and metal complexes are shown in Table 3. For the azo dye ligand, there are four stages of mass loss. The first degradation stage takes place in the range of 30-185 °C and corresponds to the loss of $C_2H_4O_2$ molecule, and represents a mass loss of 14.34 % and its calculated value was 14.93 % with Ts at 144 °C. The second degradation stage takes place in the range of 185-330 °C which corresponding to the loss of $C_2H_4O_2$ molecule and representing a mass loss of 15.39 % and its calculated value was 14.93 % with Ts at 283 °C. Finally, the third and fourth degradation stages take place in the range of 330-800 °C with Ts at 368 and 597 °C that corresponding to the loss of $C_{18}H_{10}N_4$ molecule, representing a mass loss of 70.27 % and its calculated value was 70.15 %.

The TG curves of the complexes are taken as a proof for the existing of water molecules as well as the anions to be in the coordination sphere. Decomposition of $[Cr(L)(H_2-O)Cl]Cl_2 \cdot H_2O$ complex occurs in three steps. The first and second steps occur within the temperature range of



30-270 °C with maximum temperatures 60 and 244 °C due to loss of two water and chlorine molecules. According to the literature, the azo bonds in the azo metal complexes breakdown when the temperature is higher than 260 °C [37-41]. As in the third step, the mass loss stage (270-800 °C) is due to the decomposition of a part of the ligand (C₁₂H₁₈ClN₄O_{2.5}). The remaining final product is ¹/₂Cr₂O₃ contaminated with carbon atoms.

The TG curve of [Mn(L)(H₂O)₂]Cl₂ complex showed three steps of mass loss. The first step at 30-95 °C with maximum temperature 63 °C can be attributed to loss of chlorine gas. The second and third steps at 96-800 °C with maximum temperatures 172 and 227 °C can be attributed to loss of the remaining ligand $(C_{17}H_{22}CIN_4O_5)$. The thermal decomposition of [Fe(L)(H2O)Cl]Cl2·H2O complex proceeds via five degradation steps. The first step of

complex

decomposition occurs within the temperature range from 30 to 200 °C, with a maximum temperature at 113 °C, and corresponds to the loss of one water molecule of hydration and chlorine molecule with mass loss 15.50 % (calcd. = 14.82 %). The second step of decomposition occurs in the range of 200-315 °C, with one maxima at 240 °C, and corresponds to the elimination of coordinated water molecule and chlorine gas with a mass loss of 7.90 % (calcd. = 8.90 %). The last three steps of decompositions occur within the temperature range of 315-800 °C, with three maxima at 377, 552 and 779 °C and are simultaneously decomposed to $\frac{1}{2}Fe_2O_3 + 15C$ with intermediate formation of very unstable products which were not identified. They are accompanied by a mass loss of 32.50 % (calcd. = 32.97 %) and corresponding to the loss of C₇H₁₈N₄O₃ molecule.



Fig. 3 ESR of L-Cu complex

The $[Co(L)(H_2O)_2]Cl_2$ complex is thermally decomposed in two successive decomposition steps. The first estimated mass loss of 16.48 % (calcd. = 15.67 %) within the temperature range of 30-285 °C with maximum temperature of 45 °C may be attributed to the loss of water and chlorine molecules. The final mass loss is due to the decomposition of the rest of the ligand molecule (C₂₁H₂₀N₄O₄) leaving cobalt oxide residue with contaminated carbon atom with total mass loss 84.66 % (calcd. = 84.68 %). The DTA curve of $[Ni(L)Cl_2]$ complex showed endothermic peak in the temperature range 30-140 °C which is due to mass loss of 13.50 % (calculated mass loss: 13.35 %) which corresponds to the elimination of chlorine gas. The second and third steps of the thermal decomposition, which occur in the range 140-800 °C, which are also endothermic process as shown from the DTA curve, can assigned to the loss of C21H18N4O3 molecule (observed 70.84 %: calcd. = 70.30 %) leaving NiO contaminated with carbon as residue.

The TG curve of $[Cu(L)(H_2O)Cl]Cl.H_2O$ complex indicated endothermic decompositions in four steps. The first step within the temperature range 40–135 °C with maximum temperature of 124 °C may be assigned to loss of hydrated water molecule with mass loss 3.59 % (calcd. = 3.14 %). The second and third decomposition steps occur at 135–380 °C with two maxima at 260 and 330 °C and include elimination of chlorine gas and water molecules with mass loss 15.96 % (calcd. = 15.54 %). The fourth decomposition step occurs within temperature range of 380–800 °C with maximum temperature 769 °C. This step accompanied by complete decomposition of the ligand (C₈H₁₈N₄O₃) as well as formation of copper oxide contaminated with carbon atoms as final product from which the metal content was found to be in good agreement with the data obtained from complexometric analyses.

For [Zn(L)Cl₂].H₂O complex, the TG curve showed mass loss in the temperature range of 30-290 °C (found % = 16.26, calcd. % = 16.01) associated with two DTG peaks at 47 and 192 °C. This step can be assigned to loss of chlorine gas and water molecule in two steps. On further heating, the DTG registered peaks at 311, 406 and 777 °C within the temperature range of 290-800 °C that may correspond to loss of remaining ligand fragment (C14H18N4O3) leaving ZnO contaminated with carbon as a residue. This step had mass loss of 52.45 % (calcd. = 52.16 %). The TG curve of $[Cd(L)Cl_2]$ complex showed six decomposition steps. The first stage composed of two steps (40-275 °C) and showed the removal of chlorine gas (found mass loss = 11.81 %: calcd. = 12.14 %). The other four steps (275-840 °C) represent the loss of remaining ligand (C22H18N4O3) (found mass loss = 66.0 %; calcd. = 65.98 %) with CdO residue.

Structural interpretation

From all the previous data, the newly synthesized azo dye ligand behaves as tetradentate ligand and coordinated to metal ions through two nitrogens of azo groups (N=N) and two oxygens of methoxy groups (OCH₃). The structure of the newly synthesized ligand and its metal complexes and coordination sites with metal ions can be predicted using different techniques as elemental analysis, IR, ¹HNMR, mass spectrometry, electronic spectra, magnetic susceptibility, ESR, conductivity measurements, thermogravimetric



Fig. 4 Curves of a Azo dye ligand, b L-Cr(III) and c L-Zn(II) complexes

Synthesis, spectral characterization, thermal,	anticancer and	l antimicrobial	studies of
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Table 3 Thermoanalytical results (TG and DTG) of azo dye ligand and its metal complexes

Complex	TG range/°C	DTG _{max} /°C	<i>n</i> *	Mass loss/ % found/ calcd	Total mass loss/ % found/ calcd	Assignment	Residues
L	30-185	144	1	14.34 (14.93)		Loss of C ₂ H ₄ O ₂	_
	185-330	283	1	15.39 (14.93)		Loss of C ₂ H ₄ O ₂	
	331-800	368,597	2	70.27 (70.15)	100 (100)	Loss of C ₁₈ H ₁₀ N ₄	
$[Cr(L)(H_2O)Cl]Cl_2 \cdot H_2O$	30-270	60, 244	2	17.65 (17.93)		Loss of Cl ₂ and 2H ₂ O	$\frac{1}{2}Cr_{2}O_{3} + 10C$
	270-800	455	1	49.51 (49.20)	67.20 (67.14)	Loss of C12H18ClN4O3	
$[Mn(L)(H_2O)_2]Cl_2$	30–95	63	1	7.09 (6.29)		Loss of ½Cl ₂	MnO + 5C
	95-800	172, 227	2	69.86 (70.48)	77.03 (76.77)	Loss of C17H22ClN4O5	
$[Fe(L)(H_2O)Cl]Cl_2 \cdot H_2O$	30-200	113	1	15.50 (14.82)		Loss of H ₂ O and Cl ₂	$\frac{1}{2}Fe_2O_3 + 15C$
	200-315	240	1	7.90 (8.90)		Loss of $^{1\!/_2}Cl_2$ and H_2O	
	315-800	377,552,779	3	32.50 (32.97)	56.11 (56.70)	Loss of C ₇ H ₁₈ N ₄ O ₃	
$[Co(L)(H_2O)_2]Cl_2$	30-285	45	1	16.48 (15.67)		Loss of H_2O and Cl_2	CoO + C
	285-800	661	1	68.15 (69.0)	84.66 (84.68)	Loss of C21H20N4O4	
[Ni(L)Cl ₂]	30-140	78	1	13.50 (13.35)		Loss of Cl ₂	NiO + C
	140-800	196, 255	2	70.84 (70.30)	84.43 (83.65)	Loss of C21H18N4O3	
$[Cu(L)(H_2O)Cl]Cl\cdot H_2O$	40-135	124	1	3.59 (3.14)		Loss of H ₂ O	CuO + 14C
	135-380	260, 330	2	15.96 (15.54)		Loss of Cl ₂ and H ₂ O	
	380-800	769	1	36.71 (38.08)	56.42 (56.77)	Loss of C ₈ H ₁₈ N ₄ O ₃	
$[Zn(L)Cl_2] \cdot H_2O$	30-290	47, 192	2	16.26 (16.01)		Loss of H ₂ O and Cl ₂	ZnO + 8C
	290-800	311,406,777	3	52.45 (52.16)	68.91 (68.17)	Loss of C14H18N4O3	
$[Cd(L)Cl_2]$	40-275	115, 209	2	11.81 (12.14)		Loss of Cl ₂	CdO
	275-840	289, 370, 532, 686	4	66.0 (65.98)	77.81 (78.12)	Loss of $C_{22}H_{18}N_4O_3$	

n* number of decomposition steps

analyses (TG-DTG) and X-ray powder diffraction. The proposed structures are given in Fig. 5.

Biological activities

The antimicrobial activities of the free azo dye ligand and its metal complexes are given in Table 4 which also contains standard antibiotic, amikacin for antibacterial activity and ketoconazole as antifungal agent. The data listed in this table showed that the azo dye ligand and [Mn(L)(H₂O)₂]-Cl₂ complex have no activities against all the tested microorganisms (bacteria or fungi), while the other metal complexes showed remarkable antimicrobial activities. The inhibition zone diameter (mm mg⁻¹ sample) of bacterial and fungal growth inhibition for the compounds and relation between atomic mass of metal ion and inhibition zone diameter are summarized in their graphical representations as shown in Fig. 6. On comparing the results of antimicrobial activity of the azo dye ligand and its metal complexes, it was found that Zn(II) complex showed the highest antibacterial activity against Bacillus Subtilis (Gram positive) and Escherichia coli (Gram negative), and Cd(II) complex has the highest antibacterial activity against *Staphylococcus aureus* (Gram positive), *Neisseria gonorrhoeae* (Gram negative) and *Candida albicans* (fungus). The bacterial growth inhibitory capacity of the ligand and its complexes follow the order:

$$\begin{split} &Zn(II) > Cd(II) > Co(II) > Ni(II) > Cu(II) > Cr(III) > \\ &amikacin > L = Mn(II) = Fe(III) \quad (for Bacillus Subtilis).\\ &Cd(II) > Zn(II) > Co(II) > Ni(II) > Fe(III) = Cu(II) = \\ &amikacin > L = Cr(III) = Mn(II) \quad (for Staphylococcus aureus)\\ &Zn(II) > Cd(II) > Co(II) > Ni(II) > Cr(III) = Fe(III) = Cu(II) = \\ &Cd(II) > Cd(II) > Co(II) > Ni(II) > Cr(III) = Fe(III) = Cu(II) > \\ &Cd(II) > Zn(II) > Co(II) > Ni(II) > Cr(III) = Fe(III) = \\ &Cu(II) > amikacin > L = Mn(II) \quad (for E. coli) \\ &Cd(II) > Zn(II) > Co(II) > Ni(II) > Cr(III) = Fe(III) = \\ &Cu(II) > amikacin > L = Mn(II) \quad (for Neisseria Gonorrhoeae). \end{split}$$

Higher activity of metal complex was probably due to greater lipophilic nature of the complex. It increased activity of the metal complex and can be explained on the basis of Overtone's concept and Tweedy's chelation theory [12, 42, 43]. According to Overtone's concept of cell







M = Mn(II) and Co(II).

M = Ni(II), Zn(II) and Cd(II).



$$\begin{split} \mathsf{M} &= \mathsf{Cr}(\mathsf{III}) \text{ and } \mathsf{Fe}(\mathsf{III}); \ x = 2; \ y = 1.\\ & \mathsf{Cu}(\mathsf{II}); \ x = y = 1. \end{split}$$

Table 4	Biological	activity	of	azo	dye	ligand	and	its	metal	complexes	
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Sample	Inhibition zone diameter/mm mg ⁻¹ sample									
	Gram positive		Gram negative		Fungus					
	Bacillus Subtilis	Staphylococcus aureus	Escherichia coli	Neisseria gonorrhoeae	Candida albicans					
Control: DMSO	0	0	0	0	0					
L	0	0	0	0	0					
$[Cr(L)(H_2O)Cl]Cl_2 \cdot H_2O$	9	0	9	9	0					
$[Mn(L)(H_2O)_2]Cl_2$	0	0	0	0	0					
[Fe(L)(H ₂ O)Cl]Cl ₂ ·H ₂ O	0	9	9	9	0					
$[Co(L)(H_2O)_2]Cl_2$	15	15	14	20	12					
[Ni(L)Cl ₂]	13	13	13	12	15					
$[Cu(L)(H_2O)Cl]Cl \cdot H_2O$	10	9	9	9	0					
$[Zn(L)Cl_2] \cdot H_2O$	25	20	17	22	0					
$[Cd(L)Cl_2]$	20	30	15	26	23					
Amikacin	6	9	7	6	-					
Ketoconazole	-	-	-	-	9					

permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials due to which liposolubility was considered to be an important factor that controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups [44, 45]. Further, it increases the delocalization of the π electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhanced the penetration of the complexes into lipid membrane and thus blocks the metal binding sites on enzymes of microorganisms [46]. These metal complexes also disturb the respiration process of the cell and thus





block the synthesis of proteins, which restricts further growth of the organism [47].

Anticancer activity evaluation

To study the cytotoxicity of the newly synthesized azo dye free ligand and its metal complexes, the MCF7 [8] cell line was chosen, as it is a breast cancer cell line. Figure 7 illustrated cell viability in relation to increasing concentrations of compounds. The values of concentration at which half of the maximal effect is observed (IC₅₀) with the numerical data summarized in Table 5. Cr(III) and Cu(II) complexes are inactive as they showed inhibition ratio less than 70 %, while the free azo dye ligand and other metal complexes have anti-breast cancer activity. It is observed that the ligand has the lowest activity as its $IC_{50} = 26.8 \ \mu g \ mL^{-1}$ while Zn(II) complex of the lowest IC₅₀ (12.0 μ g mL⁻¹) showed the highest anti-breast cancer activity. These results provided a perfect example of how changes in the chelation molecular structure could lead to profound differences in anticancer activity [48-50].



Fig. 7 Anticancer activity of L and its complexes

 Table 5
 Anti-breast cancer activity of azo dye ligand and its metal complexes

Complex	Surviving f	fraction/MCF7				$IC_{50}/\mu g m L^{-1}$	
	Conc./µg n	nL^{-1}					
	0.0	5.0	12.5	25.0	50.0		
L	1.00	0.77	0.69	0.51	0.44	26.8	
$[Mn(L)(H_2O)_2]Cl_2$	1.00	0.74	0.68	0.45	0.35	22.1	
[Fe(L)(H ₂ O)Cl]Cl ₂ ·H ₂ O	1.00	0.99	0.94	0.84	0.88	_	
$[Co(L)(H_2O)_2]Cl_2$	1.00	0.84	0.66	0.67	0.68	_	
[Ni(L)Cl ₂]	1.00	0.97	0.72	0.46	0.39	23.0	
$[Zn(L)Cl_2] \cdot H_2O$	1.00	0.66	0.53	0.69	0.88	12.0	
[Cd(L)Cl ₂]	1.00	1.00	1.00	0.76	0.74	_	

Conclusions

The newly synthesized azo dye ligand and its Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes are prepared and their structures are confirmed by elemental analysis, spectroscopic studies (IR, UV–Vis, ¹H NMR, mass spectrometry, electronic spectra, magnetic susceptibility and ESR), conductivity measurements, thermogravimetric analyses (TG-DTG) and further confirmed by X-ray powder diffraction. Infrared spectra discussed the chelation mode through two oxygen atoms of two methoxy groups and two nitrogen atoms of two azo groups as the ligand is tetradentate ligand. Molar conductance in DMF indicates that all complexes are electrolytes except Ni(II), Zn(II) and Cd(II) complexes are non-electrolytes. The proposed structural formulas of the above complexes are:

 $[ML(H_2O)Cl]Cl_x \cdot yH_2O \text{ where } M = Cr(III), Fe(III);$ x = 2, y = 1 and M = Cu(II); x = 1, y = 1. $[ML(H_2O)_2]Cl_2 where M = Mn(II) and Co(II).$ $[MLCl_2] \cdot yH_2O where M = Ni(II), Zn(II) and Cd(II);$ y = 0 for Ni(II), Cd(II); y = 1 for Zn(II).

The magnetic and solid reflectance measurements confirm octahedral geometry of the complexes, and XRD results showed that the free ligand and metal complexes have amorphous nature except Cd(II) complex is crystalline. ESR spectra of solid Cu(II) complex at room temperature showed axial type (d_{x2-y2}) with covalent bond character in an octahedral environment. The antimicrobial test refer that all metal complexes except Mn(II) recorded a significant antibacterial efficiency rather than the free azo ligand, but only Co(II), Ni(II) and Cd(II) complexes have antifungal activities upon the lipophilicity behaviors. Ligand and some metal complexes showed inhibitory activity against breast carcinoma (MCF7 cell line), especially Zn(II) complex showed the highest anticancer activity with low IC₅₀ values (12.0 µg mL⁻¹).

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