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Synthesis, Characterization, and Cytotoxic Activities of a Schiff Base Ligand and Its Binuclear Copper(II) and Manganese(III) Complexes

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Abstract: A novel symmetrical N_2O_2 type Schiff base (**1**) and its copper (II) (**2**) and manganese (III) (**3**) complexes were synthesized and characterized by spectroscopic, analytical, and magnetic susceptibility studies. Spectroscopic and magnetic susceptibility studies suggested that copper and manganese ions are in 2+ and 3+ states and their complexes have a binuclear double stranded helical structure in the form of 2:2 (metal to ligand) stoichiometry. Cytotoxic effects of the ligand and its metal complexes against MCF-7 (human breast cancer cell line), DLD-1 (human colorectal cancer cell line), ECC-1 (human endometrium cancer cell line), DU-145 (human prostate cancer line), MDA-MB231 (human breast cancer cell line), PC-3 (human prostate cancer line) and HEK293 (normal cells) were evaluated by determining their cellular viability using the colorimetric 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) assay. It has been found that cytotoxicity of the ligand was significantly enhanced towards cancer cells and declined towards normal HEK293 cells by metal chelation. Copper complex yielded better results in comparison with manganese complex. Particularly, copper complex showed a selective cytotoxicity, harming the cancerous cell lines while not impairing the normal cells, which is considered as the key to the future of cytotoxic therapy.

Keywords: N_2O_2 Schiff base; transition metal complexes; binuclear complexes; selective cytotoxicity; metal chelation.

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INTRODUCTION

Schiff bases have been serving as versatile and practical ligands in the field of coordination chemistry since they were synthesized for the first time by Hugo Schiff in 1864 (1). Metal complexes of Schiff bases have played a major role in the advancement of coordination chemistry whose field of application varies from physicochemical studies (2) to biological aspects (3, 4). Both symmetrical and non-symmetrical Schiff bases have been widely used as ligands to prepare metal complexes (5). Tetradentate symmetrical N_2O_2 Schiff bases and their metal complexes also contributed to the development of coordination chemistry immensely because of their structural variety, preparative accessibility, and bioactivity (6,7).

Cytotoxicity of the Schiff bases has been receiving considerable attention ever since the discovery of their effectiveness at inhibiting proliferation of cancer cells (8-11). Discovery of novel potent, selective, and less toxic anticancer agents remains one of the most active areas in the field of medicinal chemistry and drug design (12). Development of resistance by tumor cells against the existing anticancer drugs and increasing mortality rates due to cancer each year keeps this research window open for new chemotherapeutics (13). Having various biological activities despite being small molecules, transition metal complexes of Schiff bases have also aroused great attention to find new chemotherapeutic drugs. Numerous studies have reported that Schiff bases have shown mild to good cytotoxicity against various malignant tumors (14-18) and the metal included in the complex has a significant impact on the effectiveness of the compound (14,17,19-24). However, very few studies dealing with the cytotoxicity of the N_2O_2 type Schiff bases have been reported. With this in mind, we synthesized a novel symmetrical N_2O_2 type Schiff base ligand and its copper and manganese complexes and evaluated the effect of these metals on cytotoxicity against six malignant tumor cell lines and a healthy cell line. In the literature, a similar ligand and its some metal complexes have been studied regarding the structure, magnetic and luminescent properties, antimicrobial, and antioxidant activities by different groups (25-27). We have used a different aldehyde in the synthesis of the Schiff base and utilized a different synthetic approach to prepare the complexes by using different metal salts and solvent. We obtained similar binuclear double-helical structures for both complexes. Although the structural architecture of the copper complex is the same as the reported complexes in the above-mentioned studies, the structure of manganese complex is different. To the best of our knowledge, this is the first report of the double stranded helical structure for this type of manganese complex and cytotoxic activities for these types of metal complexes.

MATERIALS AND METHODS

Materials

All chemicals for the syntheses and the solvents used were of analytical grade quality from commercial sources and were used without further purification. Fourier transform infrared (FT-IR) spectra were measured with a Perkin-Elmer Spectrum Two FT-IR spectrometer in the range 4000–400 cm^{-1}. Melting points were determined on an Electro Thermal IA 9100 apparatus using a capillary tube. ^{1}H-NMR and ^{13}C-NMR spectra of the Schiff base were recorded at room temperature on a resolution Fourier transform Bruker Biospin 300 MHz spectrometer with tetramethylsilane as an internal standard. Elemental analyses of the compounds were carried out by using an LECO CHNS-932 analyzer. UV–Vis absorption spectra were measured in a 1 cm path-length quartz cell by Perkin Elmer Lambda 35 spectrophotometer in the wavelength 250–500 nm. NMR spectra of the ligand are presented in supplementary material to this paper. The mass spectra (LC-MS) were measured using an Agilent LC-MS/MS spectrometer. Magnetic susceptibilities were determined on a Sherwood Scientific Magnetic Susceptibility Balance (Model MK1) at room temperature (32 °C) using $Hg[Co(SCN)_4]$ as a calibrant; diamagnetic corrections were calculated from Pascal's constants (28).

MCF-7, DLD-1, ECC-1, DU145, PC3, and MDA-MB-231 human cancer cell lines and HEK293 cells were purchased from the American Tissue Culture Collection (ATCC, USA). The supplemented RPMI 1640 medium and DMEM: F12 were obtained from Biochrom, USA. The supplements or antibiotics such as fetal bovine serum (FBS), penicillin/streptomycin (P/S) (100 μg/mL), L-glutamine, and fluorouracil (5-FU) were purchased from Sigma-Aldrich, USA. The cells were cultured in a CO_2 incubator with 25 cm^3 tissue culture flasks (Nunc, Denmark), which was observed routinely under an inverted microscope (Olympus, Japan) for any contamination and seeded into a 96-well flat bottom microtiter plate (Nunc, Denmark). The compounds were dissolved in dimethyl sulfoxide (DMSO Molecular Biology Grade, >99.9%) from Sigma-Aldrich, USA. Absorbance was measured at 570 nm using a microplate reader (SpectraMax M5 microplate reader of Molecular Devices, USA). The cells were cultured in a tissue culture flask in 5% CO_2 incubator kept at 37 ˚C in a humidified atmosphere and observed routinely under an inverted microscope from any contaminations. Each fresh medium was replaced every 2 or 3 days until cell confluence was achieved and the cells were detached by using trypsin EDTA.

Synthesis of the ligand (1)

2-Hydroxy-6-methoxybenzaldehyde (0.31 g; 2.04 mmol) was dissolved in 15 mL of ethanol in a 100 mL round-bottom flask equipped with a magnetic stirring bar. To this solution was

added 4,4'-oxydianiline (0.204 g; 1.02 mmol) and the reaction mixture was stirred at room temperature for 2h. The excess solvent was removed with a rotary evaporator and compound **1** was obtained as yellowish solid.

Yellow solid, mp :118 °C, yield: 86%, chemical formula: $C_{28}H_{24}N_2O_5$, M_w:468.51, LC-MS(ESI):469 $[M+H]^+$, IR (υ_{max}, cm^{-1}) υ(C=N) 1615, υ(C=C) 1579, υ(C-N) 1445, υ(C-O) 1254. ^{1}H NMR (DMSO): δ 12.39 (s, 2H, Ar-O-H), 8.08 (s, 2H, Ar-CH=N), 6.03-6.60 (m, 14H, Ar-H), 2.95 (s, 6H, Ar-OCH_3). ^{13}C NMR (DMSO): δ 163.06 (Ar-C=N); 155.80 (Ar-O-Ar); 150.64 (Ar-OH); 148.09 (Ar-OMe); 143.62 (Ar-N=); 124.05, 123.29, 119.72, 119.44, 118.80, 115.63 (Ar-H); 56.05 (-OCH_3). Anal. Calcd. for $C_{28}H_{24}N_2O_5$ (%): C, 71.78; H, 5.16; N, 5.98. Found (%): C, 71.45; H, 5.23; N, 5.85.

General Procedure for the Preparation of Metal Complexes (2 and 3)

To a round-bottom flask equipped with a water condenser and magnetic stirring bar was added ligand (**1**) (0.158 mmol) and the respective metal chloride salt, MCl_2, (0.158 mmol). 20 mL of methanol was added to dissolve the reaction mixture. After refluxed for 24h, the reaction mixture was allowed to cool to room temperature. The solution was passed through a No:1 Whatman filter paper and the residue was washed with methanol and then ether. The resultant solid was dried under vacuum.

Cu(II) complex ***(2)***: Brown solid, yield: 73%, chemical formula: $C_{56}H_{44}Cu_2N_4O_{10}$, M_w:1060.08, LC-MS(ESI):1061.2 $[M+H]^+$, IR (υ_{max}, cm^{-1}) υ(C=N) 1611, υ(C=C) 1545, υ(C-N) 1459, υ(C-O) 1237. Anal. Calcd. for $C_{56}H_{44}Cu_2N_4O_{10}$ (%): C, 63.45; H, 4.18; N, 5.29. Found (%): C, 62.66; H, 4.48; N, 5.03.

Mn(III) complex ***(3)****:* Dark orange solid, yield: 67%, chemical formula: $C_{56}H_{50}Cl_2Mn_2N_4O_{13}$, M_w:1168, LC-MS(ESI):1169.1 $[M+H]^+$, IR (υ_{max}, cm^{-1}) υ(C=N) 1614, υ(C=C) 1500, υ(C-N) 1453, υ(C-O) 1231. Anal. Calcd. for $C_{56}H_{50}Cl_2Mn_2N_4O_{13}$ (%): C, 57.60; H, 4.32; N, 4.80. Found (%): C, 56.80; H, 4.71; N, 5.01.

Cytotoxicity Assay and Determination of IC_{50}

The effect of the compounds on the cellular viability was determined using the colorimetric 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) assay. This method measures mitochondrial activity based on the reductive cleavage of the yellow tetrazolium salt to a purple formazan compound by the dehydrogenase activity of intact mitochondria. Briefly, cells ($1x10^5$ cells/well) were seeded in 96-well microtiter plates (Nunc, Denmark). After exposure to various concentrations of compounds for 24 h, cells were washed once with phosphate-buffered saline (PBS) before addition of 100 µL of serum-free medium

containing 5 mg/mL of MTT (Sigma, Missouri) to each well. After incubation for 4 h, the supernatant was removed and the formazan product obtained was dissolved in 100 µL of DMSO; (Sigma). The mixture was stirred for 20 min on a microtiter plate shaker and the absorbance was read at 570 nm. Cell viability was expressed as the percentage of untreated cells that served as the control group and was designated as 100% according to the formula below.

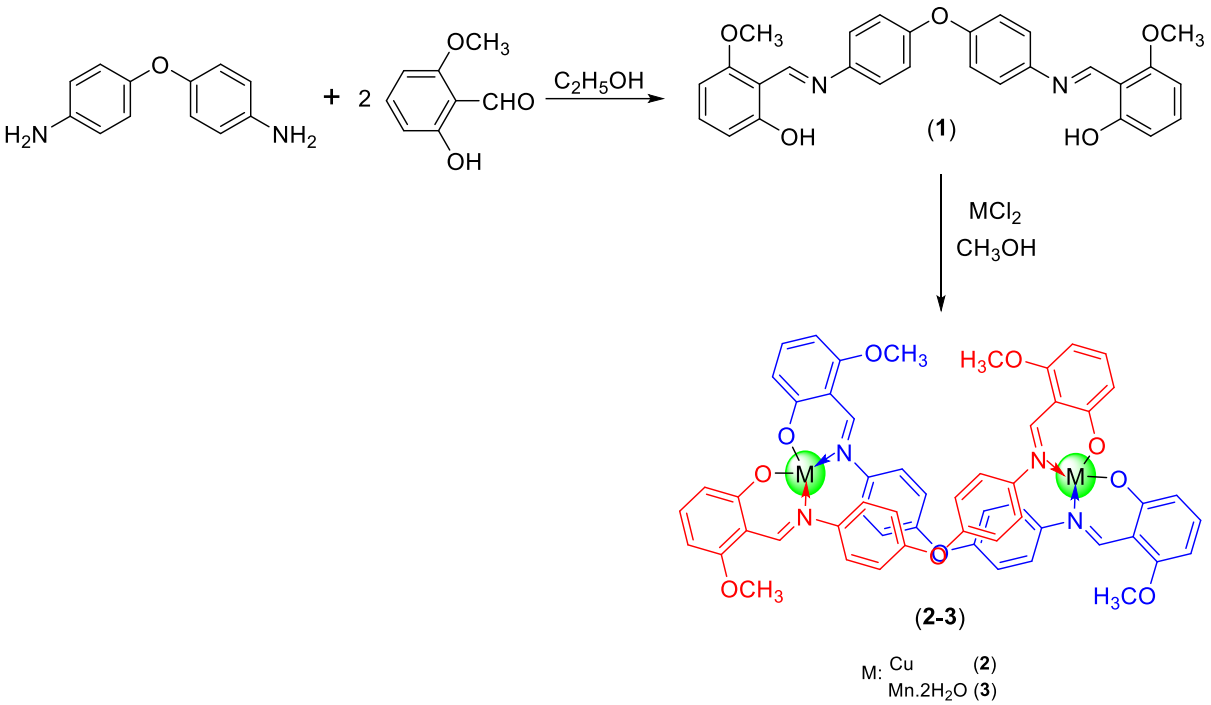
$$\%\ \text{Viable Cells} = \frac{(\text{The absorbance of the treated cells}) - (\text{The absorbance of the blank})}{(\text{The absorbance of the control}) - (\text{The absorbance of the blank})} \times 100 \quad \text{(Eq. 1)}$$

Cytotoxicity was expressed as mean percentage increase relative to the unexposed control ± SD. Control values were set at 0 % cytotoxicity. Cytotoxicity data (where appropriate) were fitted to a sigmoidal curve and a four-parameter logistic model was used to calculate the IC_{50}, which is the concentration of material causing 50 % inhibition in comparison to the untreated controls. The mean IC_{50} is the concentration of material that reduces cell growth by 50 % under the experimental conditions and is the average of at least three independent measurements that were reproducible and statistically significant. The IC_{50} values were reported at ±95 % confidence intervals (±95 % CI). This analysis was performed with Graph Pad Prism (San Diego, CA, USA).

RESULTS AND DISCUSSION

Synthesis of Schiff Base and its Metal Complexes

The Schiff base was synthesized by condensation of 2-hydroxy-6-methoxybenzaldehyde with 4,4'-oxydianiline. Copper and manganese complexes were prepared by the reaction of $CuCl_2$ and $MnCl_2$ with the Schiff base using a 1:1 ratio, respectively. The schiff base and complexes are stable in air and could be stored without any appreciable change. The complexes are soluble in THF, DMF, and DMSO. The colors of the ligand, copper, and manganese complexes are yellow, brown, and dark orange, respectively. The structures of the compounds were determined by NMR, FT-IR, UV, LC-MS, magnetic susceptibility and MM2 calculation studies.



Scheme 1. The synthetic route for the Schiff base ligand **(1)** and its copper (**2**) and manganese (**3**) complexes.

NMR Studies

^{1}H-NMR and ^{13}C-NMR spectra of the ligand are presented in supplementary data (Figures S1-S2). In the ^{1}H-NMR spectra of ligand **(1)** the phenolic -OH group of 2-hydroxy-6-methoxybenzaldeyde signal appears as a singlet at δ = 12.39 ppm. The imine protons are observed at δ = 8.08 ppm as a singlet. The phenyl protons resonate at δ = 6.03–6.60 ppm as multiplet. The O-CH_3 protons of the compound gave a singlet at δ = 2.95 ppm.

According to ^{13}C-NMR spectra, the ligand has 12 signals. The aromatic carbons are observed between 115-156 ppm. The aliphatic Ar<u>C</u>H=N-Ar and <u>C</u>H_3O carbons are seen at 163.06 and 56.05 ppm, respectively.

FT-IR, Electronic, and LC-MS Spectra

IR spectra of ligand and its complexes are given in supplementary data (Figure S3). IR spectra of the ligand and its copper and manganese complexes exhibit various bands in the 4000-400 cm^{-1} region. The absence of an O-H stretching band of the free ligand in the 3200-3800 region supports the presence of an intramolecular hydrogen bond between -OH and -C=N group. The IR spectrum of ligand shows a sharp and intense peak with the maximum at 1614 cm^{-1} which can be assigned to υ(C=N) vibration (29, 30). In the copper and manganese complexes, this band is slightly shifted to lower frequencies of 1611 and 1612 cm^{-1}, respectively, due to the coordination of nitrogen atoms in imine (-C=N) groups to the metal ion. The phenolic C-O stretching frequencies appear as strong bands at 1257

and 1196 cm^{-1} in the spectrum of the free ligand as reported for similar salen-type ligands (31,32). These bands shifted to 1242 and 1178 cm^{-1} for the copper complex and 1233 and 1172 cm^{-1} for the manganese complex. This bathochromic shift to an extend of 24 cm^{-1} in the phenolic C-O stretching vibration band confirms the coordination of the phenolic oxygen to the metal ion (33-35). A new intense band observed at 754 cm^{-1} in the spectrum of manganese complex indicates the presence of coordinated water molecules to Mn metal in the complex (36). This is further supported by the appearance of a broad OH peak between 3200-3800 cm^{-1} in the spectrum of manganese complex. The spectrum of copper complex remained unchanged in the corresponding regions confirming that no water molecule is involved in the formation of copper complex.

The electronic spectra of the ligand and its metal complexes were recorded in DMSO between 250 and 500 nm (Figure 1). UV-Vis spectrum of the free ligand exhibits two absorption peaks at 280 and 332 nm. The intense peak at 280 nm can be assigned to the $\pi \rightarrow \pi^*$ transition of the aromatic rings. The azomethine $\pi \rightarrow \pi^*$ transition band appears at 332 nm (37). The electronic spectra of the metal complexes have a similar profile. The vibrations found below 290 nm originate from $\pi \rightarrow \pi^*$ transitions associated with the phenolic chromophores whereas the absorption in the range of 300-350 nm can be attributed to $\pi \rightarrow \pi^*$ transitions of the C=N bonds. The absorption band in the region of 330 nm for Cu(II) indicates a four-coordinate square planar geometry of Cu(II) (38,39). The band in the visible region at about 360-400 nm can be attributed to $^2B_{1g} \rightarrow {}^2E_g$ transition (31). The bands observed above 400 nm for both complexes are assigned to the charge transfer transitions from the filled π orbital of the bridging phenolic oxygen atoms to the vacant d-orbital of metal ions (39).

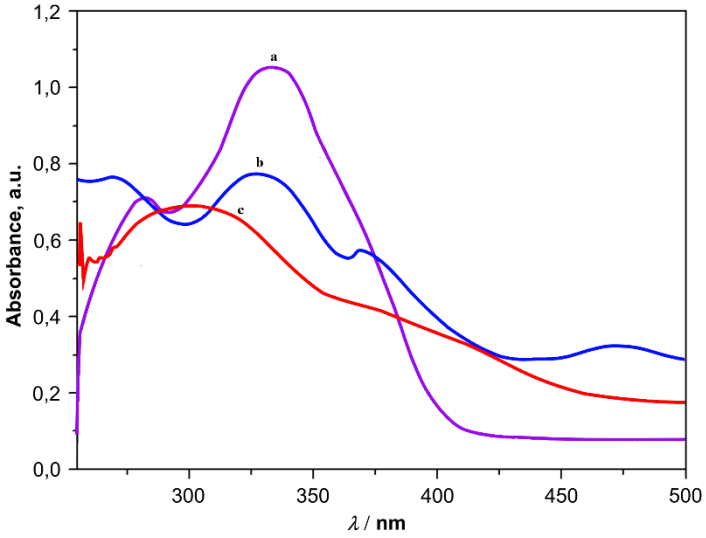


Figure 1. Electronic spectra of free ligand (a), copper complex (b), and manganese complex (c) (solution in DMSO)

The LC-MS spectra of the Schiff base ligand (**1**) and its Cu(II) and Mn(III) complexes **(2-3)** show the major fragment ions and the isotopic distributions of different intensities confirming their molecular weights. The peak at m/z=469 $[M+H]^+$ is ascribed to the molecular weight of the ligand. (Figure S4). The LC-MS spectra of the binuclear copper complex **(2)** exhibited a major peak attributed to the molecular ions at m/z=1061.20 $[M+H]^+$ for $[Cu_2L_2]$ (Figure S5). The LC-MS spectra of the binuclear manganese complex has a major peak at m/z=1169.10 $[M+H]^+$ for $[Mn_2L_2Cl_2(H_2O)_2].H_2O$ (Figure S6). These results are perfectly consistent with the calculated molecular weights of the target compounds.

Magnetic Susceptibility Studies

Magnetic susceptibility measurement provides satisfactory data to estimate the structure of the metal complexes. Magnetic moment measurements of the complexes were carried out at ambient temperature (32 °C) using $Hg[Co(SCN)_4]$ as a calibrant. Both complexes have paramagnetic characters. The magnetic moments for binuclear double-helical structures of copper and manganese complexes were found to be 2.7 B.M. and 8.1 B.M., respectively. These values are only slightly lower than the theoretical values of 2.8 B.M. and 8.9 B.M. for a binuclear Cu(II) and high spin Mn(III) ion, respectively, most probably due to a weak antiferromagnetic interaction. These results confirm that copper ion is in 2+ oxidation state with d^9 configuration having one unpaired electron and manganese ion is in 4+ oxidation state with d^4 configuration corresponding to four high-spin unpaired electrons. These data suggest that the complexes are in the form of 2:2 (metal to ligand) stoichiometry and confirm a square planar geometry around Cu ion and an octahedral geometry around Mn ion. This data is consistent with the elemental analysis results.

The structures of the complexes were further supported by the molecular mechanics (MM2) calculations. MM2 calculation indicates that the two N-donors of the ligand cannot be closer than about 9 Å which naturally means a tetradentate chelation of the ligand is unachievable. Thus, each of two ligands should bind as a bidentate ligand to two different metal centers to form a binuclear double stranded helicate complex (Figure 2). MM2 calculation also demonstrates that M-O and M-N bond distances in these dinuclear distorted double-stranded helicates are approximately 1.846 Å and 1.805 Å, respectively, which are in perfect consistency with the literature (25-27). When a tetradentate ligand fails to coordinate tetradentally to a metal atom due to a long spacer between the coordination sites, its bidentate coordination to one or two metal atoms is well known.

The structure of our copper and manganese complexes derived from bis(N-(6-methoxy-salicylidene)-4-aminophenyl)ether Schiff base could also be supported by the similar

complexes reported in the literature. Two different studies by Paula Cucos (25) and Qi-Meige Hasi (26) reported that copper complex prepared from bis(N-(3-methoxy-salicylidene)-4-aminophenyl)ether Schiff base had a binuclear double stranded helicates with copper ion in 2+ state. Our Schiff base only differs from these reported ones in that it has the methoxy groups at 6th carbons of the phenyl moieties instead of the 3rd carbons. So, it is only reasonable to expect our complex would have the same binuclear double stranded structure since the methoxy substituents are not involved in the coordination with the metal that might lead to a different structure formation. The former group also employed Co(II) and Zn(II) metals as assembling cations and found that their Schiff base formed the same complex structures with these metals as well. The latter group also prepared manganese complex of the Schiff base. Unlike the binuclear M_2L_2 type complexes formed by the metals mentioned above, the manganese metal created an ML_2 type complex and surprisingly, manganese ion was not coordinated to imino nitrogen atoms of the ligand. Instead, manganese(III) ion was coordinated to six oxygen atoms from two hydroxy and two methoxy groups of the two ligands and two water molecules. So, the manganese complex contained one manganese(III) ion, two ligands, and two water molecules. The occurrence of this phenomenon in our case is simply not possible because our methoxy and hydroxyl groups are not close enough to each other to be able to trap the manganese ion. However, methoxy and hydroxyl groups in their Schiff base were adjacent to each other.

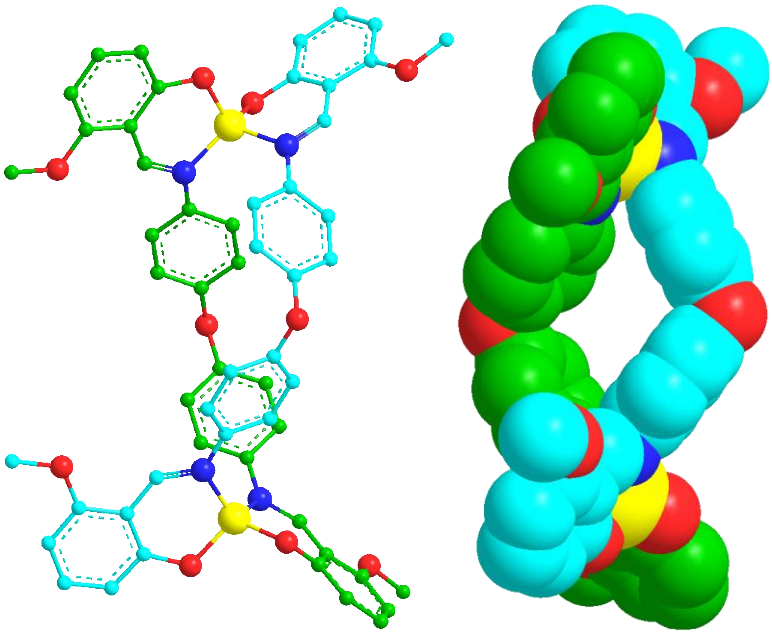


Figure 2. View of the molecular structure (left) and space-filling representation (right) of metal complexes **2** and **3** demonstrating double-stranded helical structure. Each strand is colored differently and hydrogen atoms are omitted for the clarity of the structure.

In vitro Cytotoxic Activity

Table 1. IC_{50} values for the tested cell lines following **1**, **2**, **3,** and 5-FU exposures.

Cell lines	IC_{50} values, µM.			
	1	**2**	**3**	**5-FU**
MCF-7	>1000	43.4	273.6	58,44
DLD-1	219.4	142.9	193.8	39,34
ECC-1	386.9	100	304.8	34,4
DU-145	177.2	93.5	182.9	50,22
PC3	310.5	63.2	117.3	55,44
MDA-MB231	176.6	129	187.8	62,33
HEK293	327.8	398.8	373.1	24,33

Light red, low activity; yellow, moderate activity; green, high activity.

The cytotoxic activities of **1**, **2, 3**, and Fluorouracil (5-FU) standard (positive control) against MCF-7, DLD-1, ECC-1, DU-145, PC3, MDA-MB231 cancer cell lines and HEK293 normal cell line were tested after the compounds interacted with the cells for 24 h. The results were analyzed by means of cell viability curves and given with IC_{50} values in a concentration range from 0 to 200 µM. The half-maximal inhibitory concentration (IC_{50}) is defined as the concentration required to reduce the size of the cell population by 50%. The IC_{50} values obtained for ligand and its complexes against the tested cell lines are given in Table 1 and the relation between % viable cells and the compound concentration is plotted to get the survival curve of each cell line (Figure 3).

Judging by the IC_{50} values, copper complex showed the highest cytotoxicity in comparison with the ligand and the manganese complex. Even though all the synthesized compounds showed lower cytotoxicity on cancer cells than the standard medication, 5-FU, their cytotoxicity on normal healthy cells (HEK293) is more selective than 5-FU. Especially copper complex is noteworthy as it showed close IC_{50} values to those of standard 5-FU against MCF-7, PC-3 and DU-145 and its cytotoxicity on healthy HEK293 cells is the lowest.

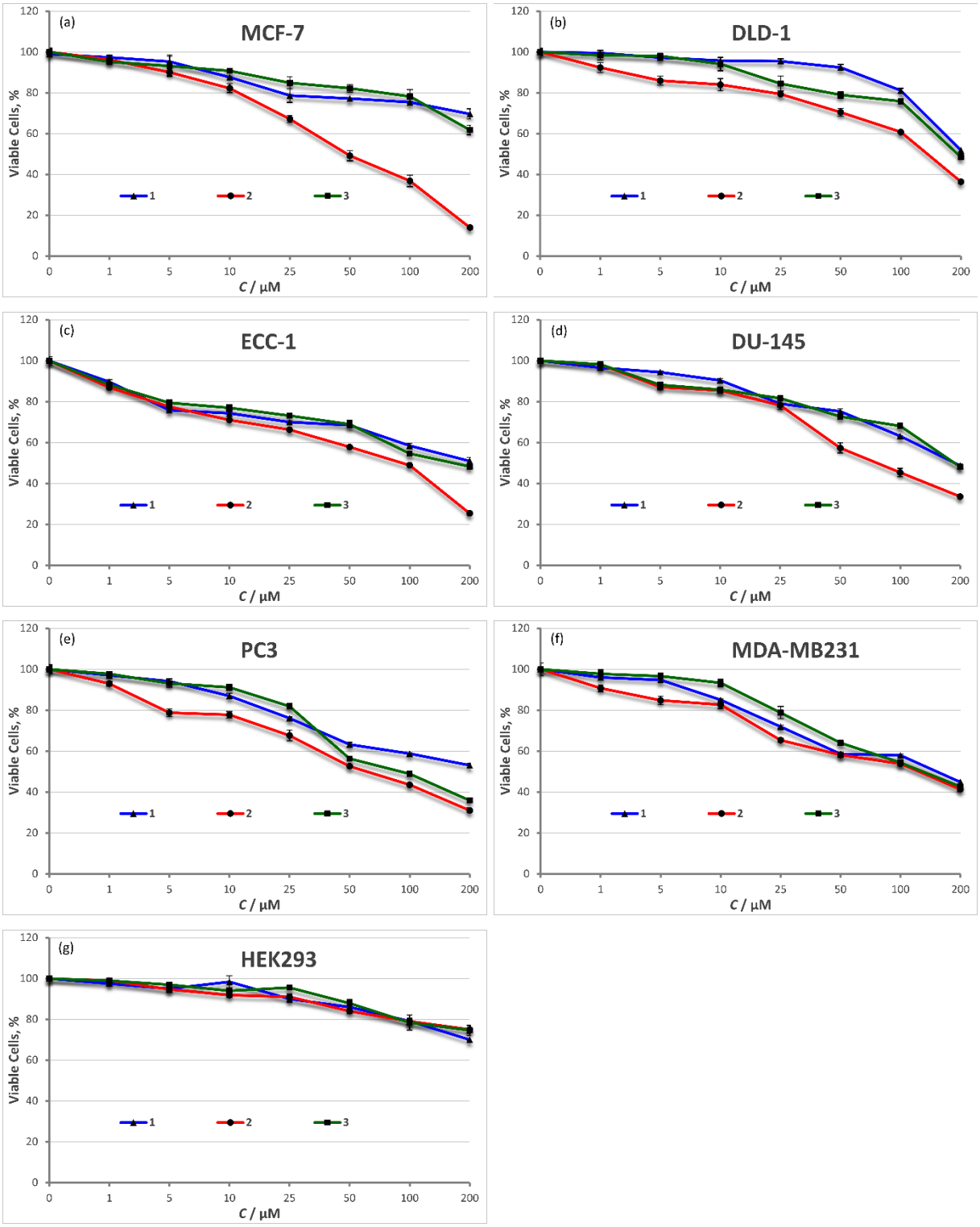


Figure 3. Plot of the % viable cells at various concentrations of **1**, **2,** and **3** against MCF-7 (a), DLD-1 (b), ECC-1 (c), DU-145 (d), PC3 (e), MDA-MB231 (f) tumor cell lines and HEK293 (g) normal cell line (Concentrations in µM).

As seen in Figure 3, the antiproliferative activity of the ligand and its complexes against the tested cells showed a dose dependent manner. Cell growth inhibition of the compounds increased gradually with the concentration increase. In our study, the results of the cytotoxic assays exhibited that the metal complexes have better bioactivity towards MCF-

7, DLD-1, ECC-1, DU-145, PC3, and MDA-MB231 cell lines in comparison with the free Schiff base ligand (**1**). The most potent effect was observed especially with Cu(II) complex (**2**). The analysis of the IC_{50} values suggests that chelation enhanced the activity against the tested malignant tumor cell lines significantly with copper ion and moderately with manganese ion. The cytotoxic enhancement was highest against MCF-7 and lowest against MDA-MB231 cells. This might be attributed to the nature of the tumor type in question. Occasionally, the response to compounds may differ from cell to cell and sometimes some tumor cells deviate from the general trend as each cancer type has different causes, morphology and metabolism.

The enhancement of the cytotoxicity towards tumor cells upon chelation might be elucidated by Overtone's concept of cell permeability (40) and Tweedy's chelation theory (41). Overtone's concept of cell permeability denotes that the lipid membrane around the cell favors the passage of lipophilic materials thus lipo-solubility is an important factor which controls the bioactivity. Also, the process of chelation dominantly affects the overall biological behavior of the compounds because chelation considerably reduces the polarity of the metal ion by partially sharing its positive charge with donor groups. Moreover, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity. This increased lipophilicity probably leads to bring down the solubility and permeability barriers of cell, which in turn enhances the bioavailability of the compounds on one hand and potentiality at another.

Interestingly, chelation did not enhance the cytotoxicity of the ligand towards healthy HEK293 cells. IC_{50} values showed that metal complexation rather slightly decreased the toxicity of the ligand against HEK293 cells. Copper complex (**2**) which had the highest cytotoxic activity towards the tested cancer cells exhibited the lowest cytotoxicity towards this healthy cell line followed by manganese complex (**3**). This selective sensitivity might be attributed to metabolic difference between healthy and tumor cells. It is well known that cancer cells use energy differently than most cell types so there are metabolic differences between healthy cells and cancer cells (42). This is very important because selectivity and good cytotoxic efficacy are some of the basic requirements of an anticancer agent. The unmanageable side effects caused by the unselectivity of most of the current chemotherapy drugs to treat cancer are a challenging and continuing problem. Many popular chemotherapeutics have good cytotoxicity but poor selectivity.

CONCLUSION

In conclusion, a novel N_2O_2 type Schiff base ligand and its Cu(II) and Mn(III) complexes were synthesized and characterized by spectroscopic methods. From the spectral and analytical data and the magnetic behavior of the complexes, the square planar and octahedral geometry have been proposed for the Cu(II) and Mn(III) complexes, respectively. Due to the long distance between the two donor sites of the ligand, a tetradentate coordination to a metal center is unattainable; thus, the ligand coordinates to two different metal atoms bidentately forming a binuclear double stranded helical structure in a 2:2 (metal to ligand) stoichiometry. This structural determination, which is perfectly consistent with the similar complexes in the literature, is further supported by the elemental analysis, LC-MS, and the magnetic susceptibility data. Cytotoxic activity studies against the tested cancerous cell lines showed that copper chelation improved the cytotoxic activity of the ligand significantly whereas Mn complexation had a slight improvement. Thus, the intensity of the enhancement is determined by the metal employed in the complex. Surprisingly, metal chelation showed just opposite effect towards healthy cells. Copper complex (**2**) which had the most potent cytotoxicity towards cancer cells showed the least detrimental effects on healthy HEK293 cells. That is, especially copper complex was found to exert a selective cytotoxicity against malignant cell lines which is considered as the key to the future of cytotoxic therapy.

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