Synthesis, Characterization and in vitro Cytotoxic Properties of Some Novel Schiff Base Metal Complexes in Hep G2 Cells

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Abstract: A novel Schiff base ligand derived from 2-hydroxy-1-naphthaldehyde and 4-methylbenzene-1,2-diamine and its transition metal complexes with Cu(II) and Zn(II) have been synthesized. The compounds were characterized by elemental analysis, IR, NMR and mass spectrometry studies. A comparative study on the cytotoxic activities of Schiff base ligands was done in HepG2 cells using MTT assay. Few of the screened Schiff bases, 2, 4 and 5 showed dose dependent cytotoxic activity, 4 being the most potent with an IC50 value of 14.32 µg/ml. 5-Fluorouracil is used as the standard reference drug. The results of the present study demonstrated that the studied compounds could indeed be the potential sources of anticancer agents and these would further enable us to evaluate their utility in biomedical field.

Key words: 2-hydroxy-1-naphthaldehyde · 4-methylbenzene-1 · 2-diamine · Schiff bases · HepG2 cell line · MTT assay · Cytotoxic activity

INTRODUCTION

Coordination compounds have always been a challenge to inorganic chemists. Today one of the most active fields of study in the vast field of inorganic research is the synthesis and characterization of complexes containing Schiff base ligands. Schiff base ligands have noteworthy importance in chemistry, exclusively in the development of Schiff base complexes, because Schiff base ligands have strong capability in forming the stable metal complexes [1, 2]. Recently, there has been a considerable interest in the chemistry of Schiff base compounds and their metal complexes due to their applications to bio-medicinal activities [3].

Human hepatic cancer is the fifth most common cancer worldwide and is responsible for more than 600,000 deaths annually [4]. The majority of patients with hepatocellular carcinoma die within 1 year after diagnosis. At present, the treatment mainly includes surgery and chemotherapy, but the curative effects of the existing chemotherapeutic drugs are not good enough and they have numerous side effects. Our understanding of the biological processes which govern carcinogenesis is growing rapidly and provides the basis for identifying novel cellular targets for anticancer drug development. Therefore, searching for highly efficient antitumor drug remains a hot research area.

Metals have been used in the treatment of diseases of humans since ancient times. The Chinese were using several elemental metals for the treatment of so many diseases since 2500 BC [5]. Recently, there has been a rapid expansion in research and development of new metal-based anticancer drugs to improve the therapeutic efficacies and to reduce general toxicity [6]. The variety of metal ion functions in biology has stimulated the development of new metallo drugs with the aim to obtain compounds acting via alternative mechanisms of action. Among the metallic compounds, copper and zinc complexes are potentially attractive as anticancer agents [6]. Since many years, many researchers have actively investigated copper and zinc compounds based on the hypothesis that endogenous metals may be less toxic. It has been established that the properties of zinc and copper-coordinated compounds are largely determined by the nature of ligands and donor atoms bound to the metal ion.

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In view of the broad spectrum of medicinal applications, especially anticancer properties of various metal based Schiff base derivatives and in continuation of our research on biologically active new molecules, we hereby report the synthesis of some new Schiff bases and their metal complexes. The newly synthesized compounds were characterized by elemental analysis, IR, $^1$H and $^13$C NMR and mass spectra. The compounds were evaluated for the possible cytotoxicity against human liver cancer (HepG2) cell lines. From the results obtained, a comparative study on the extent of cytotoxic activity of the Schiff bases and their metal complexes was done.

MATERIALS AND METHODS

General: Solvents used were analytical grades; all synthesized compounds were purified by recrystallization. Melting points were determined by using Stuart Scientific SMP1 melting point apparatus and were uncorrected. The infrared (IR) spectra were recorded on Perkin Elmer system 2000 FTIR spectrophotometer using KBr disc method. $^1$H and $^13$C spectra were recorded on Bruker 400MHz or 300 MHz spectrometer at 25°C using tetramethylsilane as an internal standard and DMSO-d$_6$ as the solvent. HREIMS spectra were obtained using a Micro TOF-Q spectrometer. Elemental analyses were performed on Perkin-Elmer series II, 2400 CHN analyzer and experimental values were within ± 0.40 % of the theoretical values. Perkin-Elmer 3000 AAS was used to determine the amount of Zn & Cu using the calibration curves of which were constructed by standard solutions. The chemicals used in this study such as, 2-nitrobenzene-1,2-diamine, methylbenzene-1,2-diamine were purchased from Acros Organics (Geel, Belgium), 2-hydroxy-naphthaldehyde and 5-bromo-2-hydroxy-1-benzaldehyde from Sigma-Aldrich.

Cell Culture Media and Reagents: RPMI 1640, trypsin and heat inactivated foetal bovine serum (HIFBS) were obtained from Gibco, UK. Phosphate buffered saline (PBS), penicillin/streptomycin (PS) solution, MTT reagent and 5-fluorouracil were purchased from Sigma-Aldrich, Germany. All other chemicals used in this study were analytical grade.

Cell Lines and Culture Conditions: Human liver cancer (HepG2) cell lines were purchased from American type culture collection (Rockville, MD, USA). HepG2 cells were maintained in RPMI 1640 containing 10% HIFBS and 1% PS. Cells were cultured in 5% CO$_2$-humidified atmosphere at 37°C.

Synthesis of Schiff-Base ligand

**Compound 1**: 4 mM 2-hydroxy-1-naphthaldehyde (0.688 g) was added to the solution of 2 mM 4-methylbenzene-1,2-diamine (0.244 g) in ethanol (30 ml) resulting in a deep yellow solution. The mixture was refluxed with stirring for 3 h. The orange solid formed was filtered and washed with ethanol. (Yield = 77%, m.p. 204°C). Fig. 1 depicts the synthetic scheme of the Schiff base (Fig. 1A) and its respective metal complexes (Fig. 1B).

Characterization of Compound 1: IR spectroscopy (KBr) 3420, 1620, 3024, 1490 cm$^{-1}$. $^1$H NMR (DMSO-D$_4$, 400 MHz): 7.03 (J=9 Hz, t, 2H, Ar); 7.23 (J=8 Hz, d, 1H, Ar); 7.36 (J=7.3, t, 2H, Ar); 7.54 (J=7.8 Hz, t, 2H, Ar); 7.66 (s, 1H, Ar); 7.70 (J=8 Hz, d, 1H, Ar); 7.80 (J=2.2, 1.4 Hz, dd, 2H, Ar); 7.90 (J=9.2 Hz, d, 2H, Ar); 8.51 (J=2.5 Hz, d, 2H, Ar) 9.66 (s, 2H, HC=N); 15.10 (s, 2H, OH) δ (ppm). $^13$C (DMSO-D$_4$, 300 MHz) δ (ppm): 119.04 (C2); 121.45 (C3); 124.41 (C4); 124.46 (C6); 129.00 (C5); 136.97 (C1); 110.02, 110.08 (C8, C9); 120.36, 120.76 (C16, C18); 122.22, 122.57 (C10, C10'); 127.68, 127.74 (C14, C14'); 128.85, 128.96 (C15, C15'); 129.83, 129.86 (C13, C13') 133.82, 133.92 (C12, C12'); 137.34, 137.70 (C11, C11'); 138.18, 138.91 (C17, C17'); 157.61, 157.85 (C9, C9'); 168.91 (C7); 169.99 (C7'); 21.59 (C18) δ (ppm).

Based on the above spectral data, the compound 1 is characterized as 2,2-(CH=CH=CH)$_2$-(4-methyl-1,2-phenylene bis (azan-1-yl-1-ylidene) bis (methan-1-yl-1-ylidene) dinaphthalen-1-ol. The structure of the compound is shown in Fig. 2.

Synthesis of Schiff-Bases Ligand Complexes

**Compound 2**: To the solution of 2 mM 4-nitrobenzene-1,2-diamine (0.306 g) in ethanol (30 ml), 0.688 g of 4 mM 2-hydroxy-1-naphthaldehyde was added which yielded an orange coloured solution. The mixture was refluxed with stirring for 1 h. Then (0.272 g, 2 mM) of zinc(II) chloride was added, followed by (500 µl, 3.6 mM) triethylamine. A red precipitate was formed. The mixture was stirred with reflux for 3 h. The red precipitate was recovered by filtration and repeated washing with ethanol (5 ml) (Yield = 78%, m. p. more than 340°C).

Characterization of Compound 2: IR spectroscopy (KBr) 3413, 1616, 626, 410 cm$^{-1}$. $^1$H NMR (DMSO-D$_4$, 400 MHz): 6.98 (J = 4.5, 4.5 Hz, dd, 2H, Ar); 7.26 (m, 2H, Ar); 7.51 (J=1.2, 7.1, 1.5 Hz, m, 2H, Ar); 7.71 (J=7.8 Hz, m, 2H, Ar); 7.83 (J=9.0, 8.99 Hz, t, 2H, Ar); 8.14 (J=2.4, 2.4 Hz, dd, 1H, Ar); 8.34 (J=9.1 Hz, d, 1H, Ar); 8.46 (J=8.5, 5.4, 8.5 Hz, 8.5 Hz,
Fig. 1A, B: The schematic representation of the synthesis of the Schiff base and its metal complexes. A) Synthesis of Schiff base ligand, B) Synthesis of Schiff base metal complexes.
Fig. 2: Chemical Structures of the synthesized Schiff base ligand (1) and its metal complexes.
1: 2,2-(1\(\text{E}\))-(4-methyl-1,2-phenylene) bis(azan-1-yl-1-ylidene) bis(methan-1-yl-1-ylidene) dinaphthalen-1-ol.
2: Aqua[2,2-[4-nitro-1,2-phenylene bis(nitromethylidyne)] dinaphthalato-\(\kappa^4\)O,N,N',O']zinc(II).
3: Aqua[4,4'-dibromo-2,2-[4-nitro-1,2-phenylenebis(nitromethylidyne)]diphenolato-\(\kappa^4\)O,N,N',O']zinc(II).
4: Aqua[4,4'-dibromo-2,2-[4-methyl-1,2-phenylenebis(nitromethylidyne)]diphenolato-\(\kappa^4\)O,N,N',O']zinc(II).
5: [4,4'-dibromo-2,2-[4-nitro-1,2-phenylene bis(nitromethylidyne)]diphenolato-\(\kappa^4\)O,N,N',O']copper(II).
6: [4,4'-dibromo-2,2-[4-methyl-1,2-phenylene bis(nitromethylidyne)]diphenolato-\(\kappa^4\)O,N,N',O']copper(II).
m, 2H, Ar); 8.88 (s, 1H, Ar); 9.87 (s, 2H, HC = N) δ (ppm). 13CNMR (DMso-D, 400 MHz): 120.80 (C3), 125.08 (C6), 129.75 (C5), 150.38 (C2), 158.55 (C4), 163.97 (C1), 110.14, 110.58 (C8, C9), 116.88, 118.38 (C10, C17), 126.52, 126.64 (C16, C16′), 128.55, 128.78 (C14, C14′), 133.45, 133.59 (C15, C15′), 136.44 (C13), 137.34 (C3), 138.19 (C12), 139.29 (C12′), 141.54 (C11), 145.70 (C11′), 147.13 (C17), 150.38 (C17′); 161.58 (C9), 161.74 (C9′); 175.39 (C7), 176.44 (C7′) δ (ppm). Elemental Analysis: calculated (found) C: 61.95 (61.55), H: 3.53 (3.53); N: 7.74 (7.69); Zn: 12.05, (12.08)%. Based on the above spectral data, the compound 2 is characterized as Aqua[2,2’-[4-nitro-1,2-phenylene bis(nitromethylidyne)]dianaphthato-k²O,N,N',O' Zinc(II)]. The structure of the compound is shown in Fig. 2.

**Compound 3:** To the solution of 2 mM 4-nitrobenzene-1,2-diamine (0.306 g) in 30 mL ethanol, 4 mM 5-bromo-2-hydroxybenzaldehyde (0.804 g) was added to obtain an orange-coloured solution. The mixture was refluxed with stirring for 1 h. Then (0.272 g, 2 mM) of zinc(II) chloride was added, followed by (500 µL, 3.6 mM) triethylamine. The mixture was stirred with reflux for 3 h. The orange precipitate formed was filtered, washed with ethanol (5 mL) and dried (yield= 73 %, m. p. more than 340°C).

**Characterization of Compound 3:** IR spectroscopy (KBr), 2974, 1613, 639, 499 cm⁻¹. 1H NMR (DMso-D, 400 MHz): 6.65 (J = 2.3, 2.3 Hz, dd, 2H, Ar), 7.31 (m, 2H, Ar), 7.62 (J = 2.7 Hz, d, 2H, Ar); 7.70 (J = 2.7 Hz, d, 1H, Ar); 8.05 (J = 2.0 Hz, d, 1H, Ar); 8.21 (J = 2.3, 2.3 Hz, dd, 1H, Ar); 8.75 (J = 2.6 Hz, d, 1H, Ar); 9.07 (s, 1H, HC = N) 9.16 (s, 1H, HC = N) δ (ppm). 13CNMR (DMso-D, 400 MHz): 103.31, 103.41 (C12, C12′), 113.22 (C10, C10′), 118.72 (C3), 121.77 (C5), 122.83 (C6), 126.56, 126.83 (C8, C8′); 137.62 (C13, C13′), 138.11, 138.41 (C11, C11′), 141.04 (C4), 146.21 (C2), 146.64 (C1), 164.46, 165.18 (C9, C9′); 172.51, 173.05 (C7, C7′) δ (ppm). Elemental Analysis: calculated (found) C: 40.00 (39.87); H: 2.18 (2.14); N: 7.00 (6.84); Zn: 10.89 (10.9) %.

Based on the above spectral data, the compound 3 is characterized as Aqua[4,4'-dibromo-2,2'-[4-nitro-1,2-phenylene bis(nitromethylidyne)]diphenolato-k²O,N,N',O′] zinc(II). The structure of the compound is shown in Fig. 2.

**Compound 4:** To the solution of 2 mM 4-methylbenzene-1,2-diamine (0.244 g) in 30 mL ethanol, 4 mM 5-bromo-2-hydroxybenzaldehyde (0.804 g), was added. A yellow color developed. The mixture was refluxed with stirring for 1 h. On adding 2 mM zinc(II) chloride (0.272 g) to the solution followed by triethylamine (500 µL, 3.6 mM), a yellow precipitate was formed. The mixture was stirred with reflux for 3 h. The yellow precipitate was obtained by filtration, repeatedly washed with ethanol (5 mL) and dried. (Yield= 86%, m. p. more than 340°C).

**Characterization of Compound 4:** IR spectroscopy (KBr), 3411, 1618, 636, 491 cm⁻¹. 1H NMR (DMso-D, 400 MHz): 2.4 (s, 3H, CH3); 6.63 (J = 1.6, 1.6 Hz, dd, 2H, Ar); 7.22 (J = 8.2, d, 1H, Ar); 7.27 (J = 3.1, 3.0 Hz, t, 1H, Ar); 7.30 (J = 3.0, 3.0 Hz, t, 1H, Ar); 7.58 (J = 2.8 Hz, d, 1H, Ar); 7.60 (J = 2.8 Hz, d, 1H, Ar); 7.72 (s, 1H, Ar); 7.76 (J = 8.4 Hz, d, 1H, Ar) 9.16 (J = 8.4 Hz, 2H, HC = N) δ (ppm). 13CNMR (DMso-D, 400 MHz): 103.31, 103.41 (C12, C12′), 117.24, 117.80 (C10, C10′), 121.90, 121.92 (C8, C8′), 126.24 (C6), 126.30 (C3), 126.36 (C5), 136.97, 137.14 (C13, C13′), 137.76, 139.74 (C11, C11′), 138.09 (C4), 138.39 (C2), 139.85 (C1), 161.93, 162.63 (C9, C9′), 171.64, 171.78 (C7, C7′); 21.92 (C14, δ (ppm). Elemental Analysis: calculated (found) C: 43.75 (44.28); H: 2.75 (2.83); N: 4.84 (4.92); Zn: 11.48 (11.5) %.

Based on the above spectral data, the compound 4 is characterized as Aqua[4,4'-dibromo-2,2'-[4-methyl-1,2-phenylene bis(nitromethylidyne)]diphenolato-k²O,N,N',O′] zinc(II). The structure of the compound is shown in Fig. 2.

**Compound 5:** To the solution of 2 mM 4-nitrobenzene-1,2-diamine (0.306 g) in 30 mL ethanol, 4 mM 5-bromo-2-hydroxybenzaldehyde (0.804 g) was added. The color of solution turned orange. The mixture was refluxed with stirring for 1 h. On adding 2 mM copper(II) chloride (0.340 g) followed by triethylamine (500 µL, 3.6 mM), a brown precipitate was formed. The mixture was stirred with reflux for 3 h. The brown precipitate was recovered by filtration, washed with ethanol (5 mL) and dried. (Yield= 77 %, m. p. more than 340°C).

**Characterization of Compound 5:** IR spectroscopy (KBr), 3367, 1606, 646, 427 cm⁻¹. Elemental Analysis: calculated (found) C: 41.37 (41.23), H: 1.91 (1.95), N: 7.24 (8.92), Cu: 10.94 (11.03) %, MS m/z: 663.4, M.W. +DMSO.

Based on the above spectral data, the compound 5 is characterized as [4,4'-dibromo-2,2'-[4-nitro-1,2-phenylene bis(nitromethylidyne)]diphenolato-k²O,N,N',O′] copper(II). The structure of the compound is shown in Fig. 2.

**Compound 6:** To the solution of 2 mM 4-methylbenzene-1,2-diamine (0.244 g) in 30 mL ethanol, 4 mM 5-bromo-2-hydroxybenzaldehyde (0.804 g) was added. A yellow colored solution was obtained. The mixture was refluxed with stirring for 1 h. On adding 2 mM copper(II) chloride (0.340 g) followed by triethylamine (500 µL, 3.6 mM), a brown precipitate was formed. The mixture was stirred with reflux for 3 h. The brown precipitate was recovered.
by filtration. The precipitate was washed with ethanol (5ml) and dried. (Yield= 90%, m. p. more than 340°C).

**Characterization of Compound 6:** IR spectroscopy (KBr) 3415, 1614, 634, 484 cm⁻¹. Elemental Analysis: calculated (found) C: 45.88 (44.24); H: 2.57 (2.13); N: 5.10 (4.89); Cu: 11.56 (11.7)%. MS m/z: 545.0 (M⁺).

Based on the above spectral data, the compound 6 is characterized as [4,4'-dibromo-2,2'-[4-methyl-1,2-phenylene bis(nitromethylidyne)diphenolato-κ²,O,N,N,O]copper(II)]. The structure of the compound is shown in Fig. 2.

**Biological Activity**

**Cells Proliferation Assay:** Newly synthesized Schiff base and its 5 metal complexes were screened for their cytotoxic activities against human hepatic cancer cell line (HepG2). Cytotoxic effect of the compounds was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [7]. HepG2 cells (100 µl cells/well, 1.5 x 10⁵ cells/ml) were inoculated in 96 wells microtitre plate. Then the plate is incubated in CO₂ incubator for over night in order to allow the cell for attachment. 100 µl of different concentrations of test substance was added into each well containing the cells. The plates were incubated at 37°C with an internal atmosphere of 5% CO₂ for 72 hr. After this treatment period, 20 µl of MTT reagent was added into each well and incubated again for 4 hr. After the incubation, 50 µl of MTT lysis solution (DMSO) was added into the wells. The plates were further incubated for 5 mins in CO₂ incubator. Finally, plates were read at 570 and 620 nm wavelengths using the micro-titer plate reader (Hitachi U-2000, Japan). 0.1% DMSO was used as negative control and 5-fluorouracil was used as positives control.

**Statistical Analysis:** The results were expressed as the mean±standard deviation (S.D) and the statistical significance was evaluated by using the student's t-test. P-values < 0.05 implied significance.

**RESULTS AND DISCUSSION**

The Schiff base ligand (1) was prepared by refluxing of 4 mM 2-hydroxy-1-naphthaldehyde with 2 mM 4-methylbenzene-1,2-diamine in ethanol. The schematic representation of the synthesis of the Schiff base and its metal complexes is depicted in the Fig. 1. The structure of the synthesized ligand was established with the help of IR, NMR and elemental analysis data. All metal(II) complexes (2)–(6) were prepared by using the respective metal salts with the Schiff base ligand. All these complexes are intensively coloured, air and moisture stable amorphous solids with high melting point. They are insoluble in common organic solvents and only soluble in DMSO. Visualization was made by using iodine vapours.

IR spectra of the synthesized Schiff base and its complexes showed the characteristic absorption band at 1600–1616 cm⁻¹ corresponding to –C=N– group in the molecule. Also the absence of carbonyl stretching band at 1700 cm⁻¹ clearly indicates amino condensation and hence the formation of Schiff bases. The ¹H-NMR spectra of all Schiff base complexes showed a singlet or doublet proton signal at the region from δ 9.0 to 9.95 due to the presence of proton in –N=CH group in the molecules, confirming the formation of Schiff base (Figs. 3 to 8).

Mass spectra of all Schiff base complexes showed molecular ion peaks which were in agreement with their molecular formula. The physical and elemental analysis data of the synthesized Schiff base metal complexes are shown in Tables 1 and 2.

Fig. 3: ¹H NMR spectrum of compound 1

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Fig. 4A, B: A) IR spectrum of compound 2, B) $^1$H NMR spectrum of compound 2
Fig. 5A,B: A) IR spectrum of compound 3, B) $^1$H NMR spectrum of compound 3
Fig. 6A,B: A) IR spectrum of compound 4, B) $^1$H NMR spectrum of compound 4
Fig. 7A,B: A) IR spectrum of compound 5, B) Mass spectrum of compound 5
Fig. 8A,B: A) IR spectrum of compound 6, B) Mass spectrum of compound 6
Fig. 9A,B: A and B) Bar graph illustrates the cytotoxic effect of Schiff base ligand and its metal complexes on proliferation of human hepatic tumor cells (HepG2). C) The dose-dependent cytotoxic effect of 5-fluorouracil on HepG2 cell line.
Table 1: Physical and analytical data of the metal complexes

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<th>Code</th>
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<th>Molecular weight</th>
<th>MP °C</th>
<th>Yield (%)</th>
<th>C</th>
<th>H</th>
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<td>C_{2}H_{2}N_{2}O_{2}Zn</td>
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<td>&gt; 340</td>
<td>78</td>
<td>61.95 (61.55)</td>
<td>3.53 (3.53)</td>
<td>7.74 (7.69)</td>
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<td>Compound 3</td>
<td>C_{2}H_{2}BrN_{2}O_{2}Zn</td>
<td>600.55</td>
<td>&gt; 340</td>
<td>73</td>
<td>40.00 (39.87)</td>
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<td>Compound 4</td>
<td>C_{2}H_{2}BrN_{2}O_{2}Zn</td>
<td>569.58</td>
<td>&gt; 340</td>
<td>86</td>
<td>43.75 (44.28)</td>
<td>2.75 (2.83)</td>
<td>4.84 (4.92)</td>
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<td>Compound 5</td>
<td>C_{2}H_{2}BrN_{2}O_{2}Cu</td>
<td>580.67</td>
<td>&gt; 340</td>
<td>77</td>
<td>41.37 (41.23)</td>
<td>1.91 (1.95)</td>
<td>7.24 (8.92)</td>
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<tr>
<td>Compound 6</td>
<td>C_{2}H_{2}BrN_{2}O_{2}Cu</td>
<td>549.70</td>
<td>&gt; 340</td>
<td>90</td>
<td>45.88 (44.24)</td>
<td>2.57 (2.13)</td>
<td>5.10 (4.89)</td>
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Table 2: Physical and IR spectral data of the metal complexes

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<td>Compound 4</td>
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<tr>
<td>Compound 5</td>
<td>Brown</td>
<td>3367, 1606, 646, 427</td>
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<tr>
<td>Compound 6</td>
<td>Brown</td>
<td>3415, 1614, 634, 484</td>
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Table 3: IC_{50} values of tested samples against HepG2 cell line

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<th>Sample Code</th>
<th>IC_{50} in µg/ml</th>
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<td>Compound 2</td>
<td>56.67</td>
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<td>Compound 3</td>
<td>74.98</td>
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<td>Compound 4</td>
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<td>Compound 5</td>
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<td>Compound 6</td>
<td>78.18</td>
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<tr>
<td>5-fluorouracil</td>
<td>4.6</td>
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In the present work, cytotoxic potencies of the newly synthesized Schiff base and its metal complexes were evaluated using MTT assay on HepG2 cell line. The results were reported as mean percentage inhibition of cell proliferation (±S.D.) when compared to that of the untreated control cells for each concentration. The IC_{50} for each compound screened was also calculated (Table 3). Among all compounds tested, compound 4 and 5 showed more potent cytotoxicity with IC_{50} (concentration of test substance to achieve 50% inhibition) 14.32 and 17.07 µg/ml respectively. Interestingly, the derivatives showed more activity than the original Schiff base ligand molecule 1 (IC_{50} 19.20 µg/ml). Results of cytotoxicity studies of synthesized compounds and the standard drug, 5-fluorouracil were illustrated in Fig. 9.

Interestingly, Schiff bases complexes 4 (with metal ion zinc and methyl substituent) and 5 (with metal ion copper and nitro substituent) were found to be active with low IC_{50} values. Preclinical studies showed that several classes of anticancer agents are able to chelate metal ions [8] have encouraged interest in metal chelating agents as promising anticancer agents. For instance, a typical chelating agent with antitumor activity is the group of bis(thiosemicarbazones), first reported by French and Freedlander [9]. The thorough studies of Petering and Petering [10] in animal model, demonstrated that the action of the bis(thiosemicarbazones) is due to the formation of Cu(II) chelates in vivo. Similarly, studies on another synthetic compound, 3-ethoxy-2-oxobutyraldehyde-bis-thiosemicarbazone, showed that the compound was found to be deprived of anticarcinogenic activity when administered to animals fed a diet low in copper. When copper was included into the drinking water at doses that had no effect upon tumor growth, the compound suppressed tumor growth in dose-dependent manner [9].

The halogen (bromine) atom might have added to the potency of the compounds as a halogen substituent produces simultaneously an increase in lipophilicity and an electron attracting effect. Thus, the anticancer activity of Zn(II) and Cu(II) complexes is probably due to the lipophilicity of the complex that facilitates the transport of Zn(II) and Cu(II) into the cell [11, 12]. Hossain et al. [13] also reported that chelation of the ligand to the metal ions increased the bioactivity of some of the compounds.

CONCLUSION

We have successfully synthesized a novel Schiff base ligand and its 5 metal complexes. In the MTT assay, the metal complexes of Schiff base, 4 and 5 showed significant cytotoxicity. The IC_{50} of 5-fluorouracil was found to be 4.6 µg/ml. Since myelosuppression, mucositis, dermatitis, diarrhea and cardiac toxicity are the adverse effects of 5-fluorouracil, there is ample scope for further study on the structural modification and mode of action of the Schiff bases complexes 4 and 5.

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