POTENTIALLY ACTIVE METAL OF COBALT, COPPER AND ZINC COMPLEXES DERIVED FROM SCHIFF BASE LIGAND OF 3-ETHOXY-2-HYDROXY-BENZALDEHYDE AND ANILINE FOR THEIR ANTICANCER ACTIVITY

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ABSTRACT

New Schiff base transition metal complexes derived from 3-ethoxy-2-hydroxy-benzaldehyde and aniline were characterized by magnetic susceptibility measurements, molar conductance, MS, FT-IR, ¹H-NMR, ¹³C-NMR, Absorbance and EPR spectra. The spectral evidences clearly showed that all the metal chelates distorted octahedral geometry except Zn(II) complex which exist square planar geometry. Electron Spin Resonance (ESR) spectrum of [CuL] complex was coinciding with proposed geometries and other reported complexes. All the synthesized compounds were studied for anticancer studies by MTT method using Hela cells. From anticancer activities, the observed IC₅₀ values of synthesized complexes are significantly higher than standard Cis-platin. The cytotoxicity of the tested compounds against the Hela cell line pursued their order Co(II) > Cu(II) > Zn(II) > ligand. Among them, Co(II) complex showed higher cytotoxic activity than other complexes and Schiff base.

Keywords: Schiff base, Metal complexes, Anticancer activity, Biological evaluation.

1. INTRODUCTION

Metal compounds have more attracted spacious attention due to their essential applications in bactericidal, anticancer and flame retardant fields [1-5]. The reasonable achievement of Schiff base metal complexes in cancer therapy has been established by Cisplatin which is tranquil one of the most effectual and best-selling anticancer drug in worldwide [6-8]. It has been broadly used as an anti-metastatic drug for various cancers since its discovery over 30 years ago which drugs are luxurious and severe toxic pains [9] for the treatment of health disorders. Therefore, it is required for the detection of metallodrugs with less or no side effects and capable of persuading apoptosis in the host cells of human cervical cancer cells. Schiff bases are usually regarded as “privileged ligands” due to easily preparation by the condensation reaction between aldehydes and imines. Schiff base is essential and pervasive ligand due to its chelated structure, synthetic flexibility, moderate electron-donor and apt structural similarity with natural bio-molecules. Their structural characteristics have been assessed by various spectral, analytical techniques and their biological activities [10-15].

The outcome of chemotherapy causes severe side effects which embrace neurotoxicity, vomiting, nephrotoxicity and ototoxicity [16, 17]. To conquer these side effects, another effort was made to reinstate those drugs with non-platinum based metal complexes. The various reports on the role of transition metals like copper in coordination chemistry have been hastily escalating due to their less toxicity and more stability. Moreover, cytotoxic effect of copper complexes was 4-50 times higher than that of other metal complexes and cisplatin which can be subjugated as a potential choice for platinum drugs [18-20]. Several studies for biological activities of Schiff base metal complexes together with their antibacterial [21-23], anticonvulsant [24], antifungal [25] and anticancer [26] have been reported earlier [27-31]. Anticancer studies are commonly observed to increase upon coordination of these ligands with transition metals such as zinc, copper, cobalt and...
nickel. This enhanced bioactivity has explained by Overton’s concept and chelation theory [32] that is based on lipophilicity and penetration of the complexes through lipid membranes. In addition, there is an urgent need to find new and improved anticancer agents to fight against diseases. Schiff bases are more significant due to their chelating ability, stability and biological applications. It is also reported that salicylaldehyde derivatives with one or more halo-atoms in the aromatic ring shows biological activities like antitumor, antibacterial and antifungal activities [33]. Presently, Metal-based drugs have gained more essential in medicinal fields which are used as medicines for the treatment of diabetes, anti-inflammatory, cancer and cardiovascular diseases [34]. The drug resistance shown by the cell upon acquaintance of antibiotic and anticancer drugs has always set a target for biochemist to look for a more potent and drug with lesser drug resistance [35].

Several methods and procedures have been fetched for the preparation of imines in the literature works. Based on it, the primary aim of our work is to synthesize Schiff base transition metal complexes derived from 3-ethoxysalicylaldehyde and aniline and its selected metal chloride salts. The spectral, analytical characterizations of synthesized metal complexes have been well studied and its anticancer studies using MTT method is more explored.

2. EXPERIMENTAL

2.1. Material and Methods

All chemicals and solvents were of analytical reagent grade and used without further purifications. Experiments were carried out at room temperature unless otherwise stated and metal (II) salts were used as chlorides. Micro analytical data (C, H, N) were collected on CHNS Perkin Elmer 2400 elemental analyzer [36]. The spectroscopic data were obtained from selected studies: UV-Visible spectra by Perkin Elmer UV-Visible spectrophotometer model lambda 25 in the range of 200-800 nm. $^1$H and $^{13}$C NMR spectra of ligand and its Zn(II) complex were recorded using NMR spectrometer model Bruker Avance (II) (400 MHz, d$_6$-DMSO solvent). Melting point of the metal complexes was noted by electro thermal 9100 apparatus using open capillaries and unconnected. TGA/DTA was done on STH6000 Perkin Elmer instrument under inert H$_2$ atmosphere. EPR spectra were recorded in DMF solution at RT (298 K) and LNT (77 K) using Bruker EPR spectrometer model EMX 10/(2) X-bond ER 4119 HS cylindrical resonator.

2.2. Synthesis of Schiff base

Compound 2-ethoxy-6-phenyliminomethylphenol (3-ESA) was synthesized according to the literature method [37, 38]. To the solution of aniline (0.913 ml), 3-ethoxysalicylaldehyde dissolved in absolute ethanol was added in an equimolar ratio (1:1). The solution was continuously stirred for 2 hours using magnetic stirrer until precipitate appeared in the reaction mixture. The crude product was filtered when the reaction completed (monitored by TLC) and re-crystallized in ethanol to give analytically pure substance (Scheme 1).

![Scheme 1: Synthesis of Schiff base from 3-Ethoxy-2-hydroxy-benzaldehyde and Aniline](image1)

![Scheme 2: Synthesis of Co(II), Cu(II) and Zn(II) metal complexes from Schiff base](image2)
2.2.1. Characterization of [2-Ethoxy-6-phenyliminomethylphenol]
The typical yield 76%; Solid; M.P.: 65°C; FT-IR (KBr, cm\(^{-1}\)): 3058 (\(\nu\text{OH}\)), 1636 (\(\nu\text{HC=N}\)), 1330 (\(\nu\text{C-O}\)); \(^{13}\)C NMR (100MH\(_z\), D\(_6\)-DMSO): \(\delta=\)116.2, 118.5, 119.2, 121.1, 123.8, 127.0, 129.4, 147.7, 148.1, 157.7; \(^1\)H NMR (400 MH\(_z\), D\(_6\)-DMSO, ppm) \(\delta=\)13.81 (S, 1H, Ar-C-O), 8.62 (S, 1H, HC=N), 6.85-7.44 (m, 8H, Ar- H); Analytical for \(C_{13}H_{13}Cl\text{NO}\): C-43.1, H-4.8, N-3.1.

2.3. Synthesis of metal complexes
To the solution of (0.05 mol) Schiff base ligand in 20 ml of hot absolute ethanol, a solution of (0.05 mol) respective metal chlorides dissolved in 20 ml of hot absolute ethanol was added and the resulting solution was refluxed for about 5 hours on mantle with water condenser until the reaction was completed [39, 40]. The red colored pure complex was obtained by slow evaporation of the solvent. The typical yield 76%; Solid; M.P.: 165°C; FT-IR (KBr, cm\(^{-1}\)): 3045(OH), 1634(C-H), 1307(C-N), 1218, 1238, 1270, 1299, 147.5, 148.2, 151.3, 164.2; \(^1\)H NMR (400 MH\(_z\), D\(_6\)-DMSO); \(\delta=\)8.84 (S, 1H, HC=N), 6.86-7.49 (M, 8H, Ar-H); Analytical for \(C_{13}H_{13}Cl\text{NO}Zn\): C-49.0, H-4.3, N-3.5; found C-47, H-4.1, N-3.4.

2.3.2. Characterization of Cu(II) metal complex
The solution was slowly evaporated to dryness at room temperature to yield a solid. The brown colored complex was obtained in ethanol by re-crystallization. The typical yield was 68%; M.P.: 250°C; FT-IR (KBr, cm\(^{-1}\)): 3045(OH), 1636 (\(\nu\text{HC=N}\)), 1307(\(\nu\text{C-O}\)), 648(\(\nu\text{C-O}\)), 417(\(\nu\text{C-N}\)); \(^{13}\)C NMR (100MH\(_z\), D\(_6\)-DMSO): \(\delta=\)117.5, 119.1, 119.7, 121.8, 124.6, 127.6, 129.9, 147.5, 148.2, 151.3, 164.2; \(^1\)H NMR (400MH\(_z\), D\(_6\)-DMSO); \(\delta=\)8.84 (S, 1H, HC=N), 6.86-7.49 (M, 8H, Ar-H); Analytical for \(C_{13}H_{13}Cl\text{NO}Zn\): C-49.0, H-4.3, N-3.5; found C-47, H-4.1, N-3.4.

2.3.3. Characterization of Co(II) metal complex
The violet coloured pure complex was obtained from re-crystallization by slow evaporation of the solution in ethanol. Typical yield was 69%; M.P.: 260°C; FT-IR (KBr, cm\(^{-1}\)): 3396(OH), 1634(C=C=N), 1301(C=O), 641(C=O), 509(C=O); analytical for \(C_{13}H_{13}Cl\text{NO}Co\): C-49.9, H-4.4, N-3.6; found: C-49.7, H-4.3, N-3.3.

2.4. Anticancer activities
The cell culture-Hela cells (Human cervical cancer cells) were cultured in liquid medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 \(\mu\)g/ml penicillin and 100 \(\mu\)g/ml streptomycin and maintained under an atmosphere of 5% CO\(_2\) at 37°C.

2.4.1. MTT Assay
The Schiff base and complexes of Co(II), Cu(II) and Zn(II) were tested for in vitro cytotoxicity using Hela cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-pentane tetrazolium bromide (MTT) assay. Momentarily, the cultured Hela cells were harvested by trypsinization and pooled in a 15 ml tube. The cells were then plated at a density of 1x10\(^5\) cells/ml cells/well (200 \(\mu\)L) into 96-well tissue culture plate in DMEM medium containing 10% FBS and 1% antibiotic solution for 24-48 hours at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the ligand and complexes in a serum free DMEM medium. Each ligand and complexes were replicated thrice and the cells were incubated at 37°C in a humidified 5% CO\(_2\) incubator for 24 h. After the incubation period, MTT (20 \(\mu\)L of 5 mg/ml) was added into each well and the cells were incubated for another 2-4 hours until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 L) was aspirated off the wells and washed with 1X PBS (200 L). Furthermore, to dissolve formazan crystals, DMSO (100 L) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC\(_{50}\) values were calculated using Graph Pad Prism 6.0 software (USA).

3. RESULTS AND DISCUSSION
The schematic preparation of the complexes is shown in scheme 1 & 2. The Co(II), Cu(II) and Zn(II) metal complexes were obtained using one mole equivalents of the Schiff base. The Schiff base selected metal complexes were well characterized using the Elemental analysis (CHN), FTIR, UV-Visible, \(^1\)H and \(^{13}\)C-NMR, EPR and TGA/DTA.

3.1. FT-IR spectra
The FT-IR spectrum of synthesized Schiff base shows band at 3416, 3058, 2977, 2927, 1640, 1330, 1247,
The band around 3416 cm\(^{-1}\) corresponds to OH stretching [41] and presence of weak band at 2977 and 2927 cm\(^{-1}\) corresponds to the C-H stretching of methyl group. IR band at 1640 cm\(^{-1}\) is due to the presence of azomethine group [42], these stretching frequencies confirmed the formation of Schiff base Ligand. IR spectrum exhibited a weak band at 1491 and 1460 cm\(^{-1}\) due to the stretching vibration of aromatic C=C. Aromatic C-H bending vibration occurred at 927 and 808 cm\(^{-1}\) respectively [43]. The absorption spectral data of the ligand at 1640 cm\(^{-1}\) indexed to imine group confirms the formation of Schiff base and the peaks appeared at 1636, 1633 and 1634 cm\(^{-1}\) in the FT-IR spectra of Schiff base complexes of Co(II), Cu(II) and Zn(II) respectively which suggested that the complexion of these metals. After the complexion, the FTIR spectrum of the prepared complexes exhibited peaks of C-O, C-N group via oxygen and nitrogen to the metal ions. However the spectrum of the free ligand shows variations corresponding to these peaks (Fig. 1).

### 3.2. UV-Visible spectra

The Schiff base ligand shows \(\pi-\pi^*\) and n-\(\pi^*\) transitions. These are also present in the spectra of the complexes and shifting to lower intensities confirms the coordination of ligand to the metal ions. The electronic spectra of the Schiff base in ethanol showed absorption bands at 220-240 nm. These UV bands are attributable to \(\pi-\pi^*\) transitions associated with azomethine group and similarly the bands at higher energy arose from \(\pi-\pi^*\) transitions within the phenyl rings. A moderately intense band observed in range of 300-330 nm is attributed to the n-\(\pi^*\) transitions of the complex. Some higher metal to the ligand charge transfer transitions probably occurred from the n-\(\pi^*\) orbitals of the Schiff base to the d-orbitals of transition metals such as Co(II), Cu(II) and Zn(II) complexes [44, 45](Fig. 2).

### 3.3. \(^1\)H and \(^{13}\)C-NMR spectra

\(^1\)H-NMR spectral data of Schiff base and Zn(II) metal complexes were recorded in DMSO-D\(_6\). The proton peak of phenolic -OH group at 13.66 had disappeared...
which suggested that the hydroxyl group coordinated to the metal centers after deprotonation [46]. The singlet at 8.62 (S, 1H) attributed to the imine hydrogen [47] in the ligand shifting to down field side in complexes clearly demonstrated that the co-ordination of azomethine nitrogen to the transitions metals. Independent assignments to the aryl protons of (3-ESA) are not possible due to overlapping of signals in this regions. $^{13}$C-NMR data showed that the signal of the imino group appears at 151ppm in agreement with data reported for analogous of Schiff base [48] and metal carbon sigma bond formation arisen when coordinated to ligand. The phenolic carbon showed that the signal at 147.51 (Ph-C-O, 1C) and the phenyl rings of ligand showed signals in between 116.23-129.44 (Fig. 3) [49].

![Fig. 3: $^1$H and $^{13}$C-NMR Spectra of (a) & (c) Schiff base and (b) & (d) Zn(II) metal complexes](image)

### 3.4. Thermo gravimetric studies
The thermal behavior of [3-ESA.ZnCl$_2$] complexes under inert N$_2$ atmosphere conditions was investigated by TGA techniques. There is a quantitative correspondence between all the curves, which exhibits a weight loss with temperature. The decomposition produced has been identified on the basis of analysis and mass spectral data. The [3-ESA.ZnCl$_2$] complex undergoes two step decomposition pattern and TGA of this complex shows first step decomposition pattern corresponding to the mass loss of 24.19% occurring in the temperature range of 100-250°C. The second step decomposition occurs at the temperature range 289.32-594.27°C with a mass loss of 64.83% due to decomposition of organic moiety. The mass of final residue corresponds to stable Zinc oxide (Fig. 4) [50].
3.5. EPR Spectra

The EPR spectrum is mainly used to analyze the paramagnetic nature of the complexes. The EPR of the synthesized complexes occurred in solid state at room temperature. The g-value for the complex [3-ESA.CuCl₂.2H₂O] at the range of 2.40 confirmed the paramagnetic nature of Cu(II) complexes [51, 52]. The observed value for the exchange interaction parameters for the Cu(II) complex [3-ESA.CuCl₂.2H₂O] suggests that local tetragonal axes are aligned parallel (or) slightly aligned and the co-paired electron is present in the dx²-y² orbital. The G values are estimated from expression: G= (g∥ - 2.0023/ g- - 2.0023). If G>L; the local axis is slightly misaligned or aligned parallel. If G<L, significant exchange coupling is present and misaligned is appreciable. EPR spectra as [3-ESA.CuCl₂.2H₂O] exhibits super-hyper fine spectrum with g∥>g, which suggests the existence of distorted octahedral geometry and the unpaired electron is predominately in dx²-y² orbital (Fig. 5).

3.6. Anticancer Activity

3.6.1. Cytotoxic activity evaluation by MTT assay

The ligand and complexes Co(II), Cu(II) and Zn(II) were tested for cytotoxicity in Hela cells (Human cervical cancer cells) by the MTT assay method [53-56]. Compounds were dissolved in DMSO and blank samples containing the same volume of DMSO were taken as controls to identify the activity of the solvent. Cis-platin was used as a standard to assess the cytotoxicity of the test compounds. The results were analyzed by means of cell inhibition expressed as IC₅₀ values and are shown in Table 1.

Table 1: IC₅₀ (g/ml) value of 3-ESA, metal complexes and cisplatin against Hela cells

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-ESA</td>
<td>188.3</td>
</tr>
<tr>
<td>Co(II) complex</td>
<td>25.51</td>
</tr>
<tr>
<td>Cu(II) complex</td>
<td>53.35</td>
</tr>
<tr>
<td>Zn(II) complex</td>
<td>55.99</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>13.00</td>
</tr>
</tbody>
</table>

The compounds exhibit cytotoxic activity at 1 µg/ml and higher concentration. Upon increasing the concentration of complexes from 1 to 500µg/ml, the % cell inhibition also increased. At 500 µg/ml, ligand, Co(II), Cu(II) and Zn(II) eliminate 67.03%, 92.41%, 76.63% and 74% of cell population respectively (Fig. 6). The IC₅₀ values of compounds against Hela cells were calculated and it was found to be 188.3 µg/ml for ligand and 25.51, 53.35 and 55.99 µg/ml for complexes Co(II), Cu(II) and Zn(II) respectively. The observed IC₅₀ values of synthesized complexes are significantly higher than standard Cis-platin. The cytotoxicity of the tested compounds against the Hela cell line follows the order Co(II) > Cu(II) > Zn(II) > ligand. Most interestingly Co(II) complex showed higher cytotoxic activity than other complexes.
Fig. 5: EPR Spectra of Cu(II) metal complex

Fig. 6: Anticancer activities of (a) Schiff base, (b) Co(II), (c) Cu(II) and (d) Zn(II) metal complexes on Hela cell line viability
4. CONCLUSION
New Schiff base metal complexes have been derived from 3-ethoxy-2-hydroxy-benzaldehyde and aniline were well characterized by suitable spectra. The analytical and spectral evidences showed that all the metal chelates are distorted octahedral except Zn(II) complex which exist square planar geometry. From anticancer activities, the observed IC_{50} values of synthesized complexes are significantly higher than standard Cis-platin. The cytotoxicity of the tested compounds against the Hela cell line pursued their order Co(II) > Cu(II) > Zn(II) > ligand. Among them, Co(II) complex showed higher cytotoxic activity than other complexes and Schiff base. The enhanced cytotoxicity in cancer cell lines with increased uptake of drug compared to the free drug and needs to be managed to meet demands and be sustainable.

Conflict of interest
The authors declare no conflict of interest.

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5. REFERENCES