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Original Research Article

Cytotoxic and anticancer studies of an oxygen and nitrogen donor novel Schiff base ligand and its copper (II) complex

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Abstract

A selected solid complex of the Schiff base ligand derived from Glutaric anhydride with Cu(II) ion was synthesized and characterized by FT-IR, Electronic, ESR Spectral Analyses, Magnetic susceptibility and Molar Conductance Measurements. The disappearance of ν(O-H) hydroxyl band of the phenolic and the lowering shift of the stretching frequency of the ν(CH=N) azomethine band in the ligand after complexation, indicated the coordination through the phenolic oxygen atom (after deprotonation) and azomethine nitrogen atom respectively of the Schiff base ligand. The lower values of molar conductance indicate the non-electrolytic nature of these complex. The ESR spectrum of the Copper complex has octahedral geometry. The Schiff base ligand and its complex further identified by ^{1}H NMR, ^{13}C NMR, SEM, EDX and molecular docking study.The anticancer potential of the Copper (II) complex was determined against A549 lung cancer cells, they exhibited appreciable anticancer activity. The *in vitro* cytotoxicity of the complex was examined against cancer cell line by MTT assay.The Schiff base Cu (II) complex was tested its cytotoxicity and found that

the 50 percentage of activity inhibitory concentration (IC_{50}) value around 91.25 percentage in 7.8 µg/ml.

Keywords : Molecular Docking study,ESR Spectral analysis, Anticancer activity,

Cytotoxicity, SEM and EDX.

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1. Introduction

The unwavering interest in the study of Schiff base compounds arises from the ease of their preparation and versatility. They are prepared from thecondensation of primary amines with aldehydes or ketones [1-2]. Schiff bases are a special class of organic compounds with varying applications in several fields of chemistry and biochemistry, especially as coordinating ligands [3-4]. Schiff base compounds and their metal complexes are very important as catalysts in various biological systems, polymers, dyes and medicinal and pharmaceutical fields[5-6]. They comprise miscellaneous therapeutically potent applications in the field of medicinal chemistry [7]. Their use in birth control, food packages and as an oxygen detector is also outlined[6]. It is noteworthy, therefore, to discuss this uncommon coordination mode as observed in the Copper(II) complex of glutaric anhydride derived Schiff base ligand. In this article, therefore, the synthesis, characterization and biological study of Cu(II) complex of some Schiff base ligand derived from condensing salicylaldehyde and 3, 4- diaminobenzophenone with glutaric anhydride. In particular, the molecular structure of the glutaric anhydride based Cu(II) complex is discussed.

2. Experimental

2.1. Methods and materials

The chemicals Salicylaldehyde purchased from LOBA Chemie Pvt. Ltd. in Mumbai, 3,4-Diaminobenzophenone, Glutaric anhydride purchased from Avra chemicals, Hyderabad and Copper nitrate hexahydrate purchased from Merck in Germany.

The infrared spectrum was recorded using KBr pellets in the range 4400-400cm^{-1}.UV-Visible spectra of the ligand and the complexes were recorded on Perkin Elmer Lambda 3B UV-Visible Spectrophotometer in the range 200-900 nm. The ^{1}H NMR spectra of the ligand and complex was recorded in Joel 500 MHz NMR spectrometer using $(CD_3)_2SO$. The molar conductance of the ligands and the complexes were measured using 10^{-3}M solution of DMSO

at 25^0C using an Elico CM-180 Conductivity meter and Elico type CC-03 Conductivity cell of cell constant 1.05 cm^{-1}. Magnetic susceptibility of Complex was measured at room temperature on a Gouy balance using $CuSO_4.5H_2O$ as a callibrant. The SEM and EDX (energy dispersive X-ray spectrometry) analyses were performed on Philips XL-30 Scanning Electron Microscope operating at 20 kV. Specimens for analysis were prepared by dusting the compounds on carbon tape.The biological importance of the synthesized ligands are assessed by performing docking studies using AutoDockVinaPyRx software [8]. The docking calculations were performed using Run Vina and the Binding affinity was used to determine the best docked structure from the output. The predicted binding affinity is in kcal/mol.

2.2. Anticancer activity (Cell line and culture)

A 549 cell line (vero line for Cytotoxicity) was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 percentage fetal bovine *serum* (FBS), penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

***2.3. In Vitro* assay for Anticancer activity: (MTT assay) [9]**

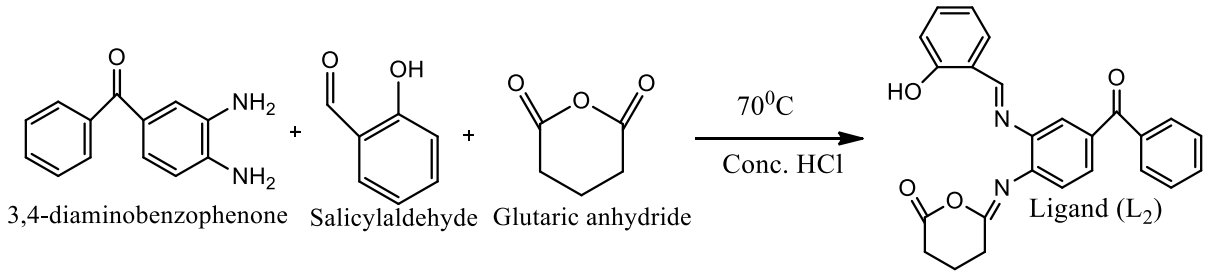
Cells (1×10^5/well) were plated in 24-well plates and incubated in 37^0C with 5 percentage CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100 µl/well (5mg/ml) of 0.5 percentage 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells .The absorbance at 570 nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50 percentage inhibition (IC50) was determined graphically. The percentage cell viability was calculated using the following formula:

2.4. Percentage Cell viability = A570 of treated cells / A570 of control cells × 100

Graphs are plotted using the percentage of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

2.5. Synthesis of (E)-6-((4-benzoyl-2-((E)(hydroxybenzylidine)amino)phenyl) imino) tetrahydro-2H-pyran-2-one (ligand)

Hot ethanolic solution of glutaric anhydride (1g, 0.0088 mole) were refluxing with salicylaldehyde (1g, 0.0081 mole). An ethanolic solution of 3, 4-diaminobenzophenone (1g, 0.0047 mole) was added in the above mixture in 1:1:1 molar ratio.Temperature was maintained at 70^0 C for 2.30 hours in the presence of concentrated hydrochloric acid. On cooling the content overnight at 0^0C. A white crystalline Schiff base Ligand ((L_2) was separated out (Scheme 1) [9-11].The synthesized compound was recrystallized from ethanol to offered pure compound (Yield- 65% andmelting point 222 - 223^0 C).



Scheme 1: Synthesis of (E)-6-((4-benzoyl-2-((E)(hydroxybenzylidine)amino)phenyl) imino) tetrahydro-2H-pyran-2-one (ligand)

2.6. Synthesis of Copper (II) complex of (E)-6-((4-benzoyl-2-((E)(hydroxybenzylidine)amino)phenyl) imino) tetrahydro-2H-pyran-2-one

Copper(II) complex was prepared using from (E)-6-((4-benzoyl-2-((E)(hydroxybenzylidine) amino)phenyl) imino) tetrahydro-2H-pyran-2-one(0.1 g, 0.003 mole) was added dropwise stirring to an ethanolic solution of Copper nitrate hexahydrate (0.05 g, 0.003 mole) then the solution was refluxed with round – bottomed flask equipped with a condenser for 2.30 hours. After completion of the reaction a dark brown coloured compound [9-11] so obtained. The Schiff base Copper (II) complex was dissolved in DMF and DMSO.(Yield - 87% and melting point >300^0 C).

3. Results and discussion

Table (1) Spectral details of (E)-6-((4-benzoyl-2-((E)(hydroxybenzylidine)amino)phenyl) imino) tetrahydro-2H-pyran-2-one (ligand)

FT-IR (cm^{-1})	**:**	3290 (-OH), 3052 (Aromatic C-H str), 1547 (-CH=N), 1730 (Alk. C=O),1648 (Ar C=O), 1514 (C=C str), 1306 (C-N), 1097(C-O).
UV-Visible (λ_{max}:nm):		267 (π-π* transition), 350 (n-π* transition)
^{1}H NMR (ppm)	**:**	10.25 (1H, s, Ar-OH), 8.20 (1H, s, CH=N), 7.04-7.80 (m, Ar-H), 3.19 (2H, t, CH_2), 2.50 (2H, m, CH_2)
^{13}C NMR (ppm)	**:**	195.9 (C=O), 158.4 (CH=N), 113.0- 132.9 (C=C), 18.5–40.5(C-C)

Table (2). Spectral details of Copper (II) complex of (E)-6-((4-benzoyl-2-((E)(hydroxybenzylidine)amino)phenyl) imino) tetrahydro-2H-pyran-2-one

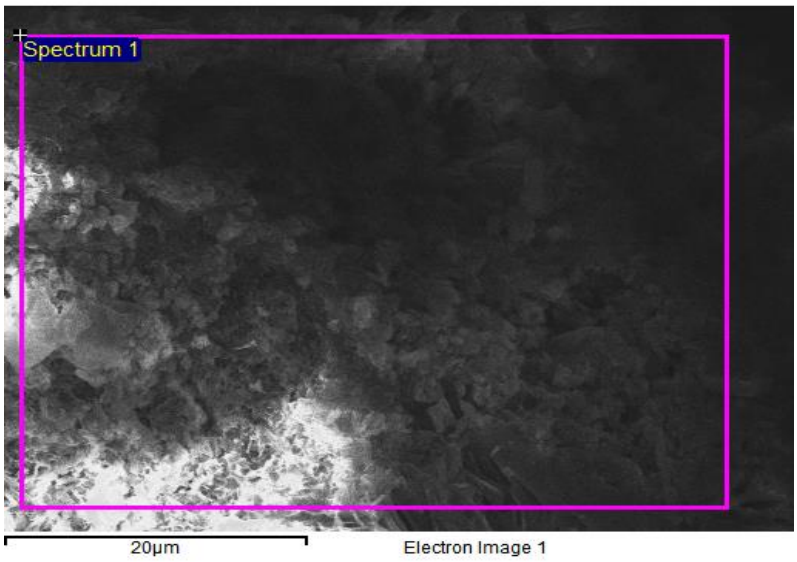
FT-IR (cm^{-1})	**:**	3398 (H_2O), 3055 (Aromatic C-H str), 1534 (-CH=N), 1648 (ArC=O), 1454 (C=C str), 1384 (N-O), 1318 (C-N), 1065 (C-O), Cu-N (470), Cu-O (526)
UV-Visible (λ_{max}: nm)	**:**	268 (π-π* transition), 385 (n-π* transition), 426 CT transition), 663(d-d transition)
^{1}H NMR(ppm)	**:**	8.12 (1H, s, CH=N), 6.90 - 7.80 (m, Ar-H), 3.3–3.9 (2H, s,H_2O) 3.10 (2H, t, CH_2), 2.31 (2H, m, CH_2), 1.81 (2H, t, CH_2)
^{13}C NMR (ppm)	**:**	195.7 (C=O), 158.3 (CH=N), 113.4- 137.2 (C=C), 16.2 – 41.3 (C-C)

The molar conductivity measurements in DMSO (10^{-3}M) at room temperature and the molar conductivity value of Cu (II) complex is 13.65$\Omega^{-1}cm^2$ mol^{-1}. The molar conductance of the complexes was found to be ranging from 14 ohm^{-1} cm^2 mol^{-1} to 22 ohm^{-1} cm^2 mol^{-1} [12]. On

the basis of molar conductance measurements of the Copper (II) complex in DMSO corresponds to be non-electrolytic in nature of the complex. The magnetic moment of the Cu (II) complex at room temperature lie in the range 1.81 – 1.83 B.M.The observed magnetic moment corresponds slightly higher value of expected the spin only value that may be explained in terms of orbital contribution and the structure might be due to distorted octahedral in nature [13].

The band for CH=N stretching of ligand was observed at lower frequency by the metal complex, indicating involvement of the azomethine nitrogen in the complex formation. The shift of the –OH band has appeared at 3290 cm^{-1} in the ligand, on complexation this band is disappear indicating deprotonation of the phenolic –OH by the complex. The presence of the absorption band at 1384 cm^{-1} in the FT-IR spectrum of the nitrato complexes suggest that both the nitrate groups are binding to the central metal ion in a primary valency. These facts are further supported by the appearance of new bands in the regions 470, 526 cm^{-1} in the Copper (II) complex, which were assigned the υ(Cu-N), υ(Cu-O) stretching vibrations respectively [14]. In the Copper (II) complex spectrum two bands at 426 nm and 663 nm may originate from the ligand to metal charge transfer transition and d-d electron transfer. The d→d transition of Cu (II) complex at 663nm. Generally Copper complexes exhibited bands in the region 512.8-563.4 nm, with a shoulder on the low energy side at ≈ 625-689.7 nm which showed that these complex are distorted Octahedral [15-16].

The ESR spectral study provides information of the metal ion environment. For the Copper (II) complex, the g value was of 2.2357 (expected 2.07 to 2.3) and the magnetic moment value was 1.82 (expected 1.70 to 1.99) [17-18]. These values clearly indicate the paramagnetic nature of the Copper (II) complex. The scanning electron micrographs of Copper (II) complex have shown in Figure(1). The distinct morphology of Schiff base-metal complex can be observed. This complex showed less porosity when compared with the other complexes. Figure (2) showed the EDX spectrum of Copper (II) complex of (E)-6-((4-benzoyl-2- ((E) (hydroxybenzylidine) amino) phenyl) imino) tetrahydro-2H-pyran-2-one. EDX analysis is a non-destructive technique performed to confirm the presence of the Copper in the complex. The spectrum shows a peaks associated with Cu, C, N, O and H atoms are only observed. The results by EDX indicated that there was no contamination. The percentages of elements present in the complex are in agreement with the proposed structure.



Figure(1). SEM image of Copper (II) complex of (E)-6-((4-benzoyl-2-((E) (hydroxy benzylidine)amino)phenyl)imino)tetrahydro-2H-pyran-2-one

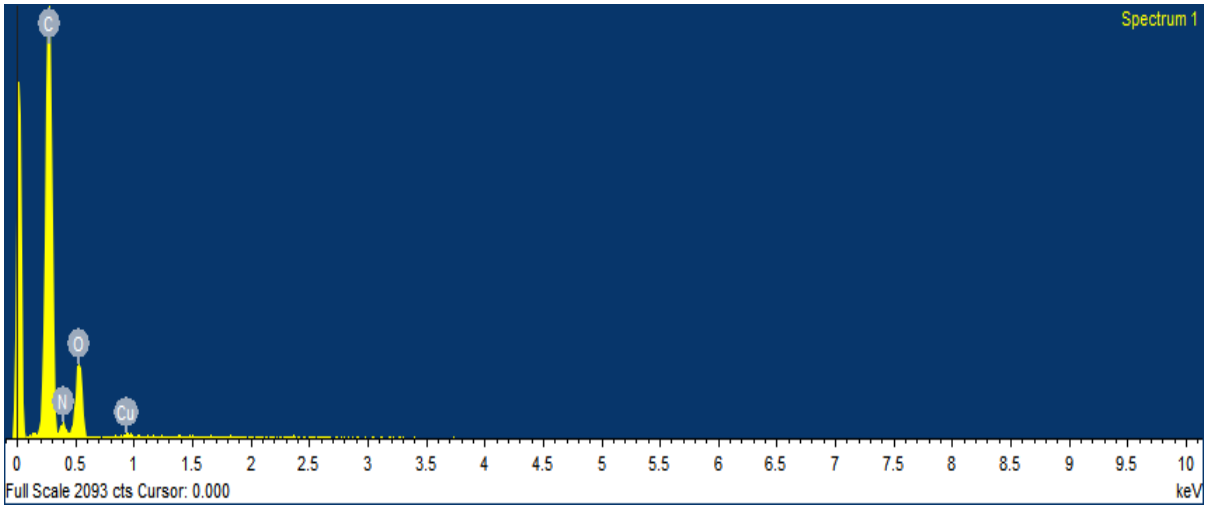


Figure2: EDX spectrum of Copper (II) complex of (E)-6-((4-benzoyl-2-((E)(hydroxybenzylidine)amino)phenyl)imino)tetrahydro-2H-pyran-2-one

Figure (3). showed human serum albumin docked with (E)-6-((4-benzoyl-2-((E)(hydroxybenzylidine)amino)phenyl) imino) tetrahydro-2H-pyran-2-one (L_2). The docked ligand (L_2) interacts with the protein by forming four hydrogen bonds with the residues Lys195 *Å*, Asp451 *Å*,Arg222 *Å* and Lys 92 *Å* with bond distances 3.12 *Å*, 3.49 *Å*, 3.21 *Å* and 3.36 *Å* respectively. The hydrogen bond showed in yellow line.

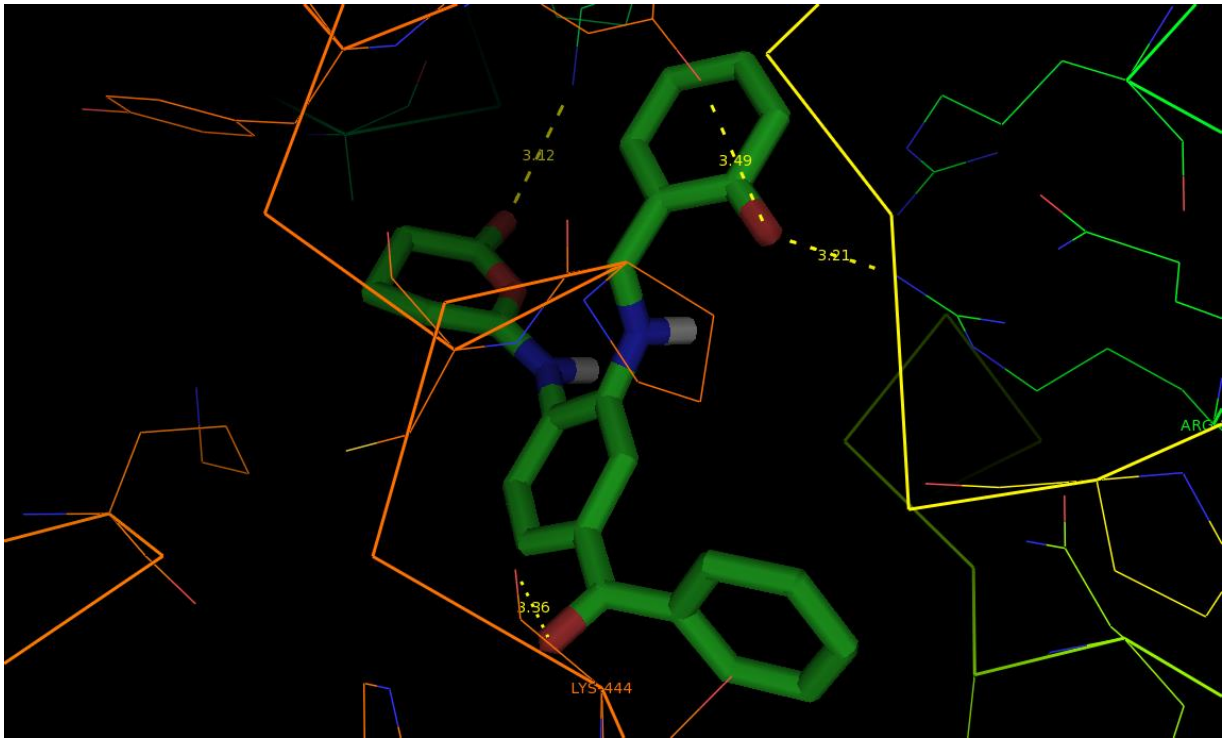


Figure3: Ligand docked with 4s1y showing formation of hydrogen bond and distances

Copper (II) complex of ligand forms three hydrogen bonds with Asp108, Asn429 and His146 residues with bond distances 3.22 *Å*, 3.11 *Å* and 3.22 *Å* respectively. Cobalt complex docked with human serum albumin which exhibited in Figure(4).Binding affinity of Copper complex (-10.7 kcal/mol) more than that of ligand (-9.2kcal/mol),which predicts a good inhibition of human serum albumin.

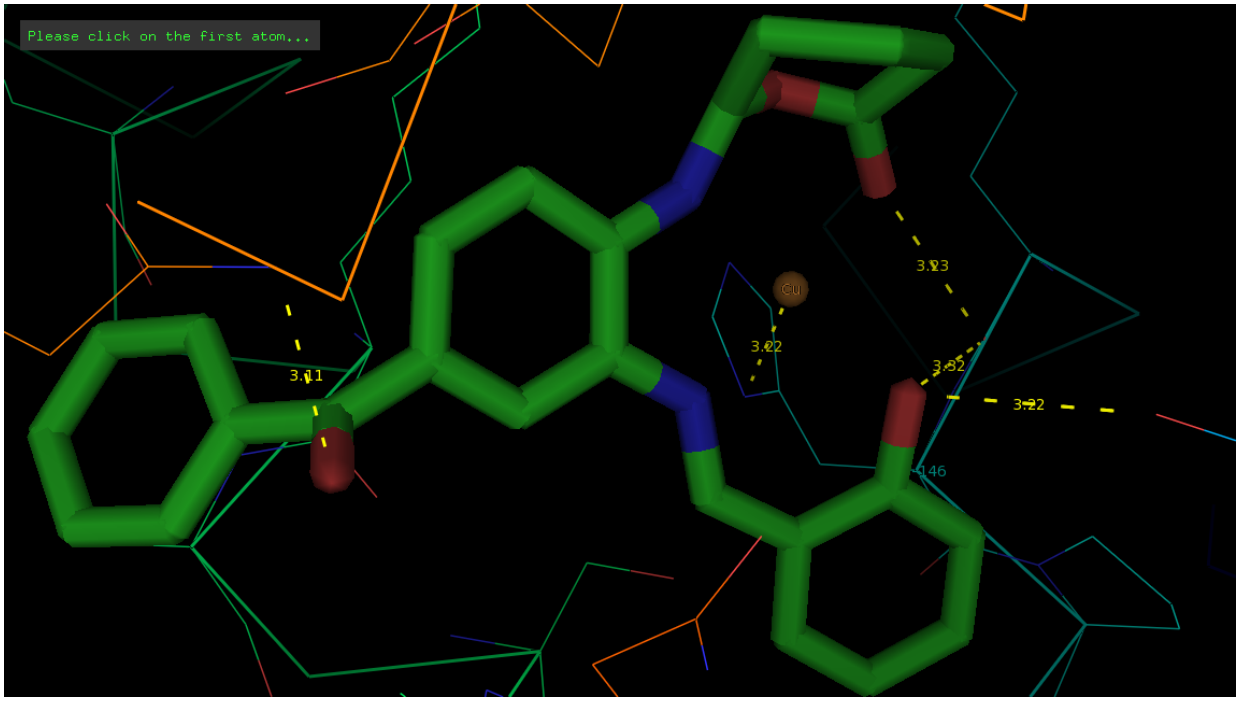


Figure4: Copper (II) complex of Ligand docked with 4s1y showing formation of hydrogen bond and distances

4. Biological Activities

4.1. Anticancer activity

The anticancer potential of the Schiff base ligand and their metal complexes were determined against A549 lung cancer cells line.Figure(5).described the percentage cell viability of Copper (II) complex of ligand Based on the MTT assay among various concentration of Copper complex with 7.8, 15.6, 31.2, 62.5, 125µg/ml found to be 68.38, 59.73, 49.58, 40.93 and 31.71 % cell viability. However, on increasing the concentration to 250, 500, 1000 µg/ml found to have 23.99, 15.39 and 6.17 % respectively, suggesting that Copper (II) complex of 250, 500, 1000 µg/ml tends to nontoxic in nature. Themorphological images of Copper (II) complex have shown in Figure(6).

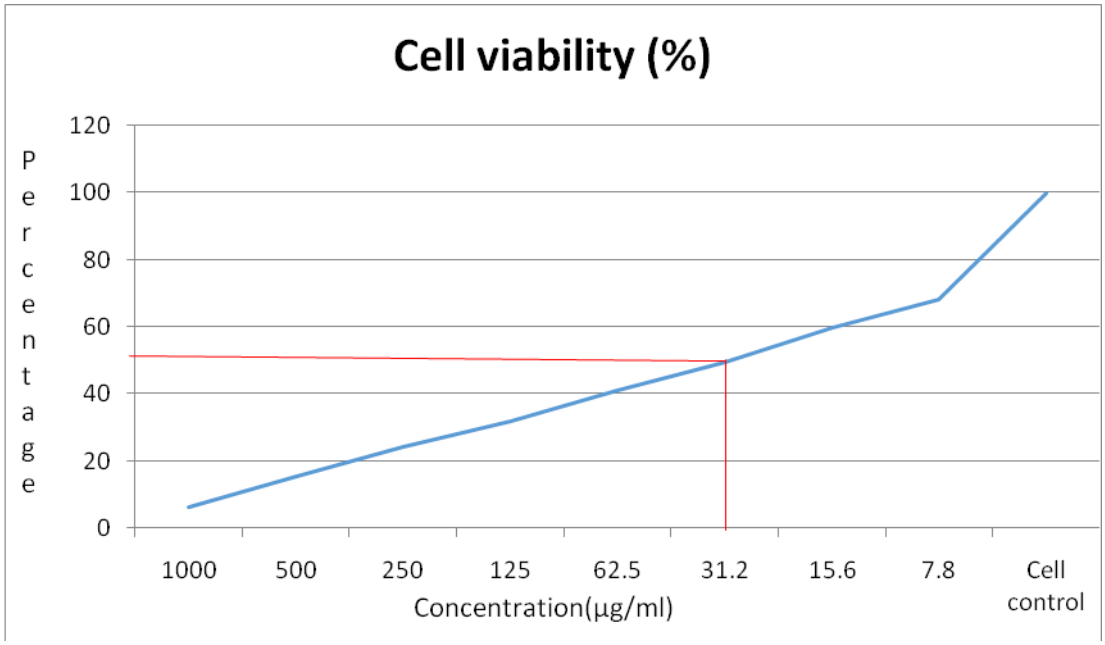


Figure5: IC50 Values of the Copper (II) complex of ligand against A549 cell line

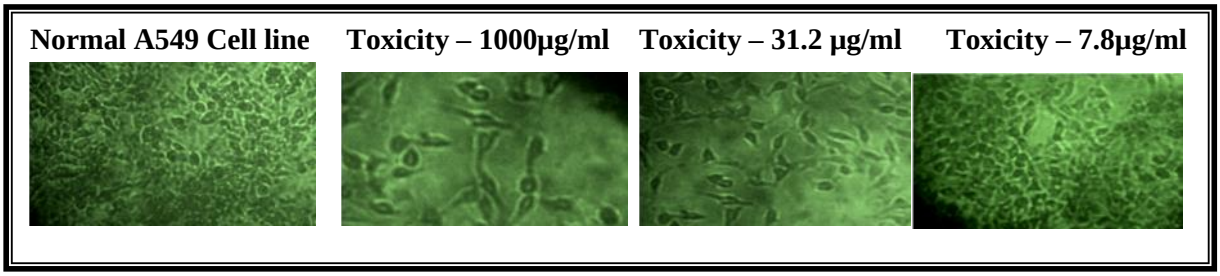


Figure6: Morphological images of Anticancer effect of Copper (II) complex of ligand on *A549 cell* line

4.2. Cytotoxicity

Cytotoxic Studies (MTT assay) on Schiff base Copper (II)complex have been listed in Fig 5.25 – 5.24. According to ISO 10993-5, on the cell viability versus toxicity among the cytotoxicity of medical devices. As per this, 80% of cell viability after the treatment may be considered as non-toxic, 79-60% mild toxic activity. Similarly, 59-40% of activity considered as moderate toxicity. However, below 40% of cell viability is considered as strongly toxic to the cell. In general, cytotoxic activity is inversely related to the antioxidant activity [19].

The Cytotoxic Cell viability of Copper (II) complex of Schiff base ligand has observed 91.25, 84.13, 77.51, 70.29, 63.47, 56.35, 49.84 and 42.52 % at different concentrations 7.8, 15.6, 31.2, 62.5, 125, 250, 500 1000 µg/ml (Figure(7). The Copper complex are non-toxic in nature

and cytotoxic property. The morphological images of Copper (II) complex cytotoxicity effect at various concentration shown in Figure(8).

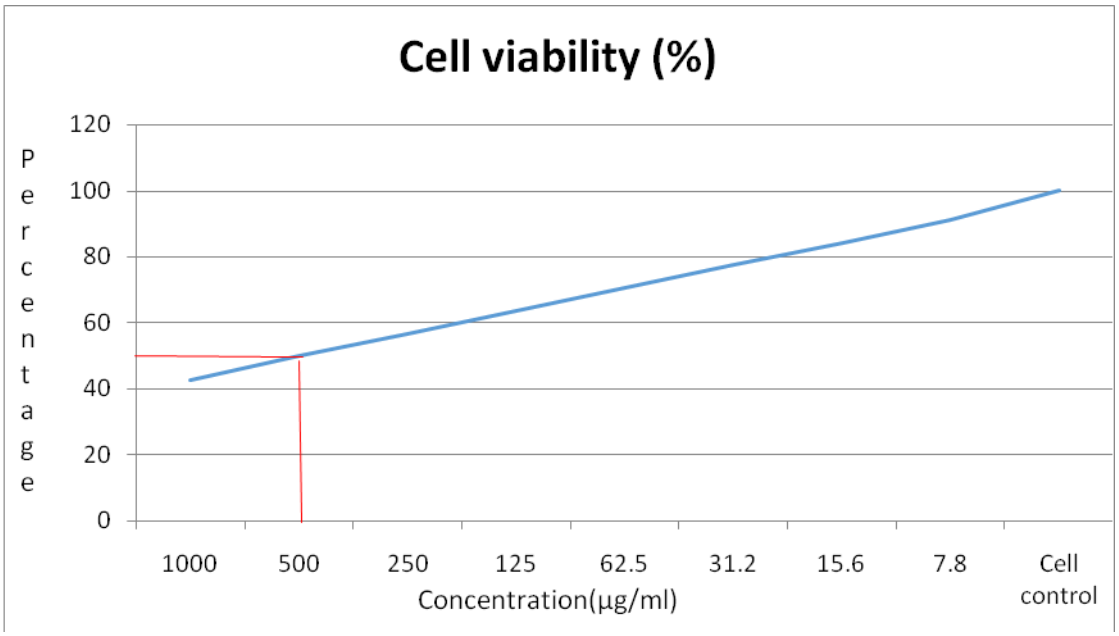


Figure7: IC50 Values of the Copper (II) complex of ligand on Vero cell line

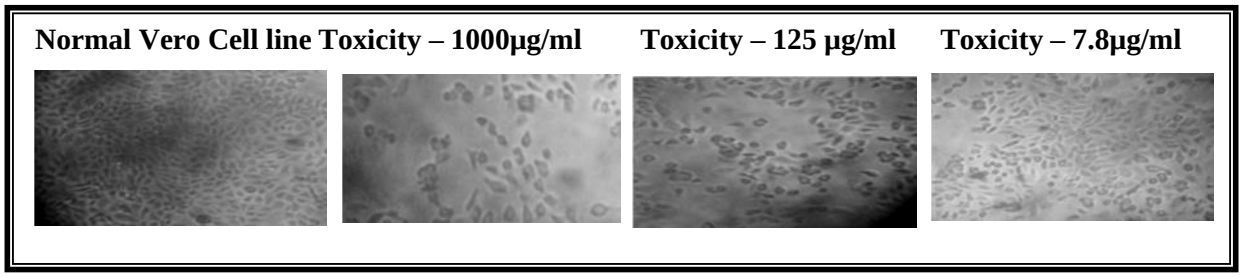


Figure8: Morphological images of Cytotoxicity effect of Copper (II) complex of ligand on *Vero cell* line

5. Conclusion

A new distorted octahedral Glutaric anhydride based Cu(II) complex wasprepared and fully characterized. Our results showedgood inhibition of human serum albumin. The binding affinity of complex is more than that of ligand. From the Anticancer and Cytotoxicity studies a Schiff base Copper complex shows a non-toxic in nature.

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