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SYNTHESIS, CHARACTERIZATION, DOCKING, ANTICANCER AND ANTIMICROBIAL STUDIES OF NOVEL SCHIFF BASE LIGAND DERIVED FROM SALICYLALDEHYDE AND ITS NICKEL (II) COMPLEX

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ABSTRACT

A new series of Co(II), Ni(II), Cu(II) and Zn(II) complexes of the Schiff base derived from Vanillin and acetoacetanilide with ethylenediamine has been synthesized. A new series of Ni(II) complex of the Schiff base derived from salicylaldehyde and 3,4-diaminobenzophenone with acetyl acetone have been synthesized. These compound have been characterized by molar conductivity measurements, infrared, electronic spectra. The Schiff base ligand and its complex further identified by 1H NMR, 13C NMR and molecular docking study. The anticancer potential of the Nickel (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 Ni(II) complex was tested its cytotoxicity and found that the 50 percentage of activity inhibitory concentration (IC50) value around 40.47 percentage in 1000 µg/ml. Antimicrobial activity of the Schiff base ligand and their metal complex reveals that the Schiff base transition metal complex show significant activity against some bacteria. model and EAC cell induced ascites tumour model were mainly used for antitumor studies. Copper complex administrated at different concentrations in Swiss albino mice to access increases in the survival rate and the life span of Ascites tumour enduring mice in a concentration dependent manner. Antimicrobial activities of the Schiff base ligand and their metal complexes reveals that the Schiff base transition metal complexes show significant activity against some fungi and bacteria.

KEYWORDS: Docking study, anticancer activity and antimicrobial activity.

1. INTRODUCTION

Schiff bases composed of N_2O_2 donor atoms are important chelating ligands for designing supramolecular synthons, medicinal and catalytically useful metal complexes.,[1-3] There has been enormous report directed towards the development of novel chemical compounds able to arrest or reverse the development of cancer.[4-5] Biological activities of transition metal complexes derived from Schiff base ligands are one of the most exhaustively studied topic in coordination chemistry. Furthermore, nickel is an important transition metal and its coordination compounds display interesting binding properties with proteins and nucleic acids.[6] The 'N' and 'O' containing Schiff base ligands and their Nickel (II) complexes have become important due to their wide biological activity.[7-9]

The nickel (II) complex with N_2O_2 donor macrocyclic Schiff base ligand derived from 3, 4-diamino benzophenone with the acetyl acetone and Salicylaldehyde in same molar ratio have been developed using simple condensation process. This paper concentrates on the synthesis and biological activity of Schiff bases ligand and their Nickel (II) complex.

2. EXPERIMENTAL SECTION

2.1. Reagents

The important chemicals used in the present study are given in the **Table 1**. All chemicals employed in the present study were of analytical grade. Solvents employed were either of 99% purity or purified by standard laboratory procedures.[10]

Table 1: Important chemicals with specified make.

Name of Reagents	Manufacturer
Acetylacetone, Salicylaldehyde	LOBA Chemie Pvt. Ltd., Mumbai
3,4-Diaminobenzophenone	Avra Synthesis Pvt. Ltd., Hyderabad
Nickel (II) nitrate hexahydrate	Merck, Germany

2.2. Analytical methods

A variety of physico-chemical methods have been employed to characterize the structure of organic Schiff base ligands and their metal complexes and in biological studies. A brief account of these methods is given below.

2.3. Physical methods

The infrared spectrum was recorded using KBr pellets in the range 4400-400 cm^{-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 ^1H 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 EDS (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.[11] 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. Antimicrobial activity was investigated against four bacterial species. (i) Gram-positive bacteria: *Staphylococcus aureus, Enterococci* and (ii) Gram-negative bacteria: *Escherichia coli, Pseudomonas aeruginosa*.

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.

***In Vitro* assay for Anticancer activity: (MTT assay)[12]**

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.

Percentage Cell viability = A 570 of treated cells / A 570 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.

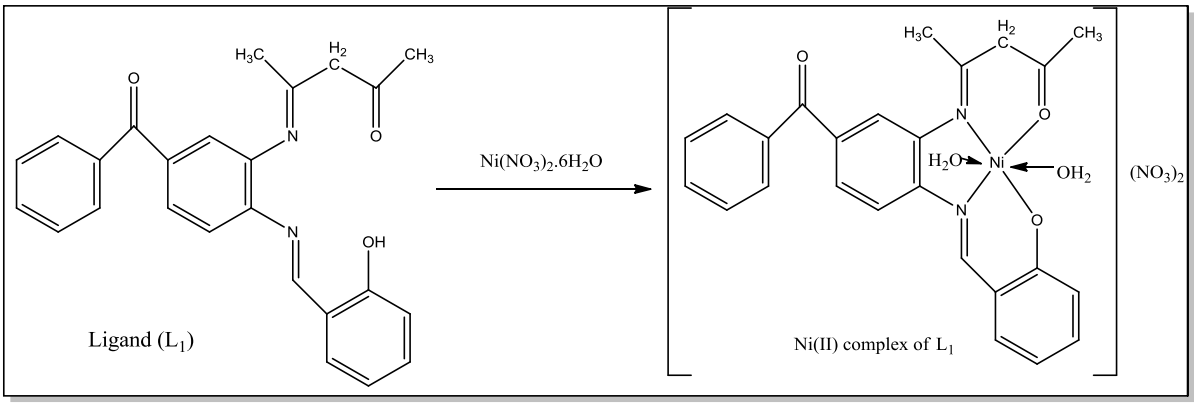
3.1. Synthesis of Schiff Base Ligand (E)-4-((5-benzoyl-2-((E)-(2-hydroxybenzylidene) amino)phenyl)imino)pentan-2-one

The new schiff base ligand was synthesized by following the literature procedure.[12-14] acetyl acetone, salicylaldehyde (1 mol) and 3, 4-diaminobenzophenone (1 mol) were added into 20 mL of absolute ethanol containing a few drops of Conc. HCl in a 100 mL round bottomed flask. The reaction mixture was refluxed for 2.30 hrs. It was then cooled and ice-cold water was added. The product so formed was filtered, washed, dried and recrystallized from alcohol, Yield 68%. Analysis: ($C_{23}H_{20}N_2O_2$). Calculated mass 398.16, observed mass 398.00. ^1H NMR: 10.30 ppm (1H, s, Ar-OH), 8.10 ppm (1H, s, CH=N), 7.03-7.81 ppm (7H, m, Ar-H), 3.20 ppm (2H, s, CH_2), 2.11, ppm (3H, s, CH_3). ^{13}C NMR: 200-210 ppm (C=O), 158 ppm (CH=N), 113.0- 138.4 ppm (Ar-C). FT-IR, KBr pellets (υ, cm^{-1}): 3291 υ (-OH str sharp band); 1712 υ (C=O for acetyl acetone); 1607 υ (C=N str); 1332, 3070 (CH-Ar str); Electronic spectrum: Band absorbed around 252 and 345 nm.

3.2. Synthesis of Schiff Base Ni (II) Complex

In Scheme 3, to a solution of Schiff base ligand (L_1) (0.1 g, 0.003 mol) in ethanol was added a solution of nickel nitrate hexahydrate (0.07 g, 0.003 mol), refluxed for 2.30 hours. After refluxing the mixture was filtered while hot. The filtrate was cooled for one day around 0^oC. A brown black product was obtained[15], washed with ethanol and then dried. The percentage of yield was 80. ^1H NMR: 8.03 ppm (1H, s, CH=N), 7.1-7.8 ppm (7H, m, Ar-H), 4.50 ppm (2H, s, H_2O) 3.15 ppm (2H, s, CH_2), 1.98, ppm (3H, s, CH_3). ^{13}C NMR: 195.64 ppm (Ph-CO-Ph), 157.9 ppm (CH=N), 112.0- 138.0 ppm (C=C). FT-IR, KBr pellets (υ, cm^{-1}): 3320 υ (broad band in H_2O), 1652 υ (C=O for benzophenone); 1573 υ (C=N str); 1383 (N-O

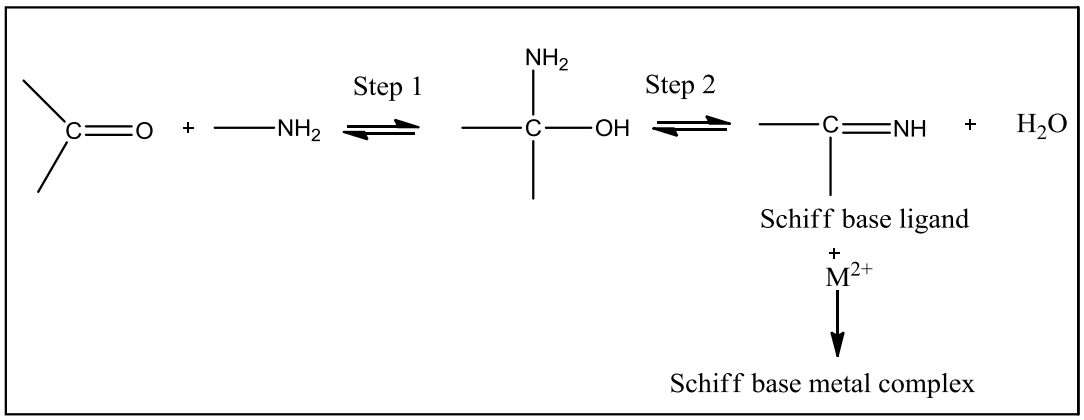
str), 478 υ (Ni-N), 534 υ (Ni-O); Electronic spectrum: Band absorbed around 258, 335, 415 and 776 nm.



Scheme 2: Synthesis of nickel (II) complex of (E)-4-((5-benzoyl-2-((E)-(2-hydroxybenzylidene) amino) phenyl) imino) pentan-2-one.

4. RESULTS AND DISCUSSIONS

In the present investigation Schiff base macrocyclic ligands and their nickel (II) complex have been synthesized via condensation of aldehydes or ketones with primary amines (Schiff et al., 1864). The general mechanism have exhibited in **Scheme 1**.



Scheme 1: Mechanism of Schiff base ligand and their metal complex formation.

The first step in this reaction is an attack of nucleophilic nitrogen atom of amine on the carbonyl carbon, resulting in a normally unstable carbinolamine intermediate. The carbinolamine loses water by either acid or base catalyzed pathways. Since the carbinolamine is an alcohol, it undergoes acid catalyzed dehydration. Typically the dehydration of the carbinolamine is the rate-determining step of Schiff base formation and that is why the reaction is catalyzed by acids. Yet the acid concentration cannot be too high because amines are basic compounds. If the amine is protonated and becomes non-neucleophilic, equilibrium is pulled to the left and carbinolamine formation cannot occur. Therefore, many Schiff base synthesis are best carried out at mildly acidic condition. The Schiff base ligand refluxed with various metal ion to form metal complexes

The synthesized complexes are soluble in DMF and DMSO. The molar conductivity measurements in DMSO (10^{-3}M) at room temperature and the molar conductivity value of Ni(II) complex is 9.80 $\Omega^{-1}cm^2$ mol^{-1}.[16] The molar conductance of the complexes were found to be ranging from 14 ohm^{-1} cm^2 mol^{-1} to 22 ohm^{-1} cm^2 mol^{-1}.[17] On the basis of molar conductance measurements of the nickel(II) complex in DMSO corresponds to be non-electrolytic in nature of the complex. The magnetic moment of the Ni(II) complex at room temperature lie in the range 2.96-2.98 B.M. The observed magnetic moment corresponding to two unpaired electrons in tune with a high spin configuration and presence of an octahedral environmental around the nickel ion in the complex.[18] Infrared spectra of ligand and metal complex are almost same with slight shift in peak position and varied intensity confirming the coordination of ligand to metal ions.

The band for C=N stretching of ligand was observed at lower frequency by 20 - 40 cm^{-1} in the metal complex, indicating involvement of the azomethine nitrogen in the complex formation. The shift of the –OH band has appeared at 3291 cm^{-1} in the ligand, on complexation this band is disappear indicating deprotonation of the

phenolic –OH by the complex. Weak bands at 500 - 550 cm^{-1} indicate Ni-O bond and a band in the region 430 – 490 cm^{-1} is due to Ni-N bond.[19] The electronic spectrum of nickel (II) complex. The two band maxima of ligand are observed between 252 nm and 345nm due to $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$.[20] On the other hand the band corresponding to azomethine showed a slight shift to longer wavelength on going from ligand to complex, indicating coordination of ligand to metals through the azomethine moiety. The electronic spectrum of Ni (II) complex exhibit four absorption peaks. The first one is 258 nm might be due to $\pi \rightarrow \pi^*$ similarly, $n \rightarrow \pi^*$ and charge transfer spectra have been reported at 335 nm and 415 nm respectively. The fourth band found at 776 nm can be focused to d-d transition.[21] The result of the finding exhibited octahedral geometry was found to Nickel (II) complex.

Table 2: Physicochemical and analytical data of the synthesized compounds.

Compound	Mol. Wt.	M. P.(in ^{0}C)	Yield (%)	Color
Ligand	398	237	68	White solid
Nickel (II) Complex	616	>300	80	Brownish black solid

4.1. Molecular docking studies

The pdb structure 4s1y[22] of human serum albumin is used for docking studies which plays a key role in increasing the growth and productivity of cells and increases overall cell health. The best docked complex selected has a binding score of -9.5 for nickel (II) complex of ligand which predicts a good inhibition. The docked ligand (L_1) interacts with the protein by forming three H bonds with the residues Ser192 and Glu292 with bond distances 3.33 *Å* and 3.53 *Å* respectively (**Fig 1**). Nickel (II) complex of ligand (L_1) forms 2 H bonds with Lys195 *Å* and Ala191 *Å* residue with bond distance 3.20 *Å* and 3.35 *Å* (**Fig 2**).

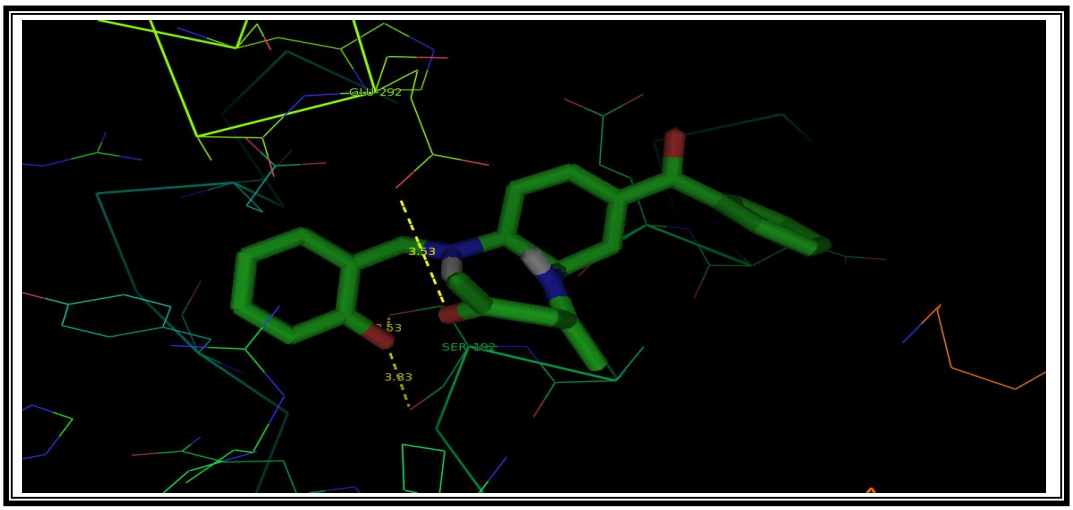


Fig 1. Ligand (L_1) docked with 4s1y showing formation of hydrogen bond and distances.

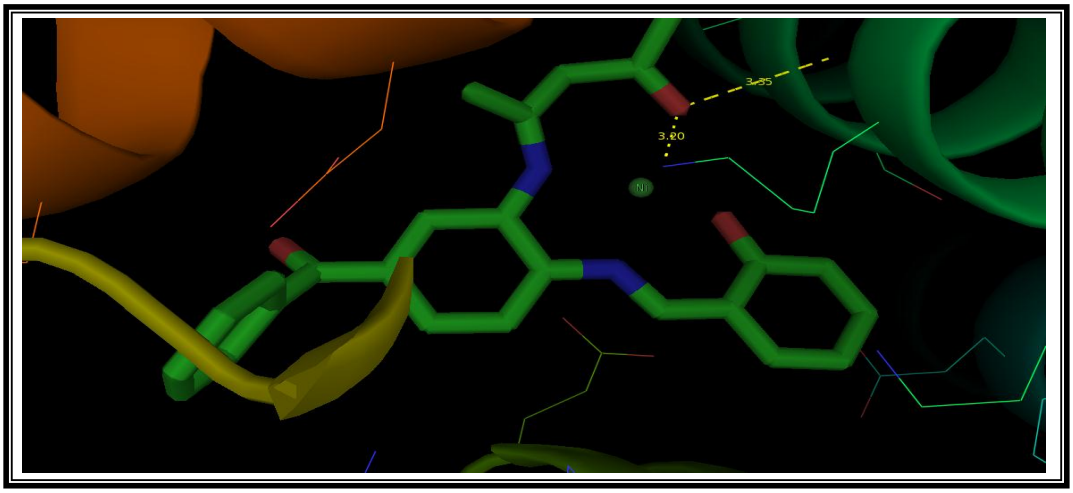


Fig 2. Nickel (II) complex of Schiff base Ligand docked with 4s1y showing formation of hydrogen bond and distances.

4.2. Antimicrobial studies

Antibacterial potential of ligand with metal complex were assessed in terms of zone of inhibition of bacterial growth. The minimum inhibitory concentrations (MIC) results for the Schiff bases were presented in **Table 3.** Ligand are significantly active against all the tested microorganisms. Growth of bacterial pathogens on different concentration (10, 25, 50, 100 µg/ml) was checked to determine the minimum concentration that inhibits the growth of the organism.

The coordination of metal ion to the Schiff base influence the magnetic property and conductance which may also be a cause for the extensive biological characteristics of the complex. The azomethine bond also extend contribution for activity of the complex.[23] The synthesized macrocyclic nickel (II) complex showed good antimicrobial activity. The ligand was more active than the metal complex[24] against all bacteria strains with the activity recorded for the complexes varying with metal ion present.

Table 3: Diameter of zone inhibition (mm) for the Schiff base ligand and its Ni (II) complex.

Compound	*Escherichia coli*			*Staphylococcus*			*Enterococci*			*Pseudomonas*		
	25	50	100	25	50	100	25	50	100	25	50	100
Ligand	-	-	9 mm	8 mm	10 mm	11 mm	4 mm	7 mm	13 mm	-	-	-
Nickel (II) Complex	-	5 mm	8 mm	6 mm	8 mm	10 mm	7 mm	9 mm	12 mm	-	-	7 mm

4.3. Anticancer Activity

The anticancer potential of the Schiff base metal complex of ligand was determined against A549 lung cancer cells. The complex of Ni(II) exhibited appreciable anticancer activity. The percentage of cell inhibition in different concentration of Nickel (II) complex have shown in the **Fig 3** and **Table 4**. The *in vitro* cytotoxicity was examined against cancer cell line by MTT assay. The morphological image of Nickel (II) complex was display in **Fig 4**. It may be accomplished that the nickel complex possess considerable anticancer activity for cancer cell line.

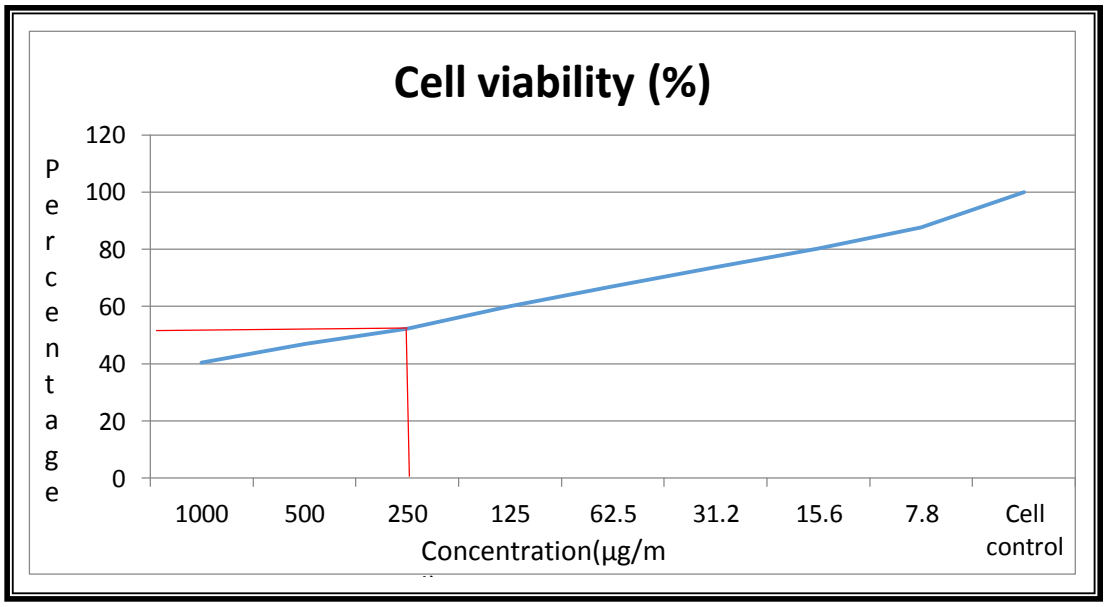


Fig 3. IC50 Values of the Nickel (II) complex of ligand against A 549 cell line.

Table 4: Anticancer effect of Nickel (II) complex of ligand on A 549 cell line.

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.786	40.47
2	500	1:1	0.911	46.91
3	250	1:2	1.016	52.31
4	125	1:4	1.166	60.04
5	62.5	1:8	1.303	67.09
6	31.2	1:16	0.434	73.84
7	15.6	1:32	1.559	80.27
8	7.8	1:64	1.703	87.69
9	Cell control	-	1.942	100

Consider that the standard / cell control (A 570) inhibition activity is considered as 100% with proportional to cell viability. Lower the concentration of the Nickel (II) complex found to have better activity with perfect to cell viability. Upon increasing the concentration by double the concentration of Nickel (II) complex if 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/ml the cell viability found to be gradually decreases, (i.e) 80.27, 73.84, 67.09, 60.04, 52.31, 46.91 and 40.47 respectively. Hence, the lower concentration of Nickel (II) complex have exhibited better anticancer activity than that of their higher concentrations.

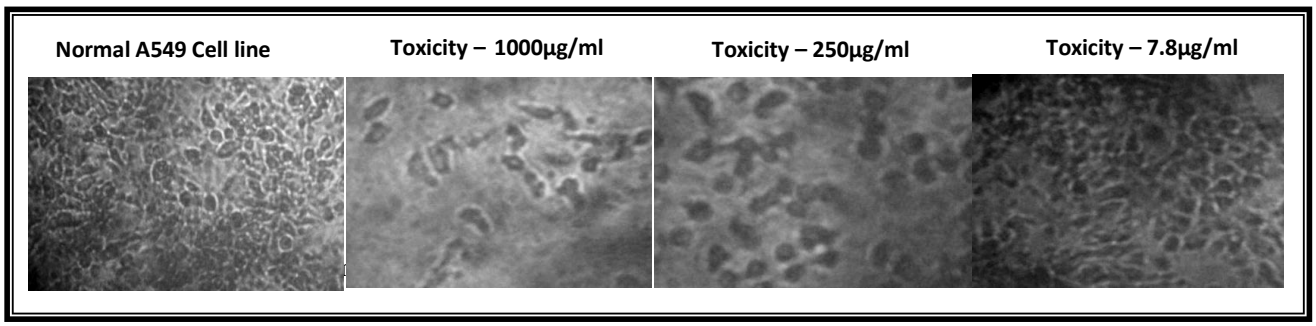


Fig. 4 Morphological image of anticancer effect of Nickel (II) complex of ligand on A549 cell line.

CONCLUSIONS

The formation of mononuclear Nickel (II) complex are thermally stable. The complex was characterized by spectral and analytical data. Based on the data, an octahedral geometry has been assigned to the Nickel complex. The antimicrobial studies carried out with the complex and ligand. These confirm that they are good antimicrobial agent, inhibition levels were determined. From the anticancer study the Schiff base Nickel (II) complex better anticancer activity in the low concentration.

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