

## Synthesis, Characterization, Bioactivity and Cytotoxicity of NS Schiff base and its Ni (II), Cd(II) and Zn(II) metal complexes

Isam Hussain T. Al-Karkhi\*    Abeer Khalid Yaseen    Eman Turkey Shamkhy

Department of Basic Science- College of Dentistry- University of Baghdad- Bab Al-Moazam- Baghdad - Iraq

### Abstract

A new nitrogen sulphur Schiff base has been prepared from the condensation 1-(pyridin-4-yl)ethanone with 2-methylbenzyl hydrazinecarbodithioate. The ligand was reacted with Ni(II), Cd(II) and Zn(II) metal salts to form new coordinated complexes. The new 2-methylbenzyl (2Z)-2-[1-(pyridin-4-yl)ethylidene]hydrazinecarbodithioate ligand and the new metal complexes were characterized *via* various physico-chemical and spectroscopic techniques and based on the data analysis the metal complexes are expected to be octahedral in geometry. The Schiff bases and their complexes were screened for antimicrobial and cytotoxic activities. [Cd(NS)<sub>2</sub>] displays strong activity against the bacteria strains and cytotoxicity while the other compounds shows moderate and weak activity.

**Keywords:** ligand, pyridine, ethanone, metal complexes

### Introduction

Metal chelates of and their Schiff bases have been of great interest to researchers since the last century when it was found that pyridine complexes displayed carcinostatic activity in the leukemia <sup>[1]</sup>. These complexes have also been known to possess a wide range of anticancer <sup>[2]</sup>, antibacterial <sup>[3-5]</sup>, antifungal <sup>[6-8]</sup>, antiamebic <sup>[9]</sup> and insecticidal activities <sup>[2-5]</sup>. It was reported here in the synthesis, characterization and biological studies of the new 2-methylbenzyl (2Z)-2-[1-(pyridin-4-yl)ethylidene]hydrazinecarbodithioate ligand and its metal complexes.

### Experimental

All chemicals and solvents were of analytical grade and were used as received. **Preparation of 2-methylbenzyl hydrazinecarbodithioate** <sup>[9]</sup> Potassium hydroxide (0.2 mol) was dissolved in absolute ethanol (70 ml). To this solution, hydrazine hydrate (0.2 mol) was added and the mixture was cooled in an ice-salt bath to 0°C. Carbon disulphide (0.2 mol) was added dropwise with constant stirring over a period of one hour. The two layers that subsequently formed were separated using a separating funnel. The light-brown lower layer was dissolved in 40% ethanol (60 ml) below 5°C. The mixture was kept in an ice-bath and to it, 3-methylbenzyl chloride (26.5 ml, 0.2 mol) was added dropwise with vigorous stirring of the mixture.

The sticky white product, (S3MBDTC), which formed was filtered and left to dry overnight in a desiccator over anhydrous silica gel. (Yield: 75%, m.p. 135°C)

**Preparation of 2-methylbenzyl-[1-(pyridin-4-yl)ethylidene]hydrazine carbodithioate** 2-methylbenzyl hydrazinecarbodithioate (0.01 mol) was dissolved in hot acetonitrile (100ml). This was added to an equimolar solution of 1-(pyridin-4-yl)ethanone in ethanol (10 ml). The mixture was heated and stirred for 30 minutes and then allowed to stand for a few hours, after which yellow crystals formed, which were filtered off and recrystallised from acetonitrile. Yields were fairly high, ca. 85%, C: 59.23(60.29), H: 5.54(5.43), N: 12.65(13.32) and S: 22.03(20.33) **General method of synthesis of the transition-metal complexes** The transition metal salts used were the acetate salts of Ni(II), Cd(II) and Zn(II) (0.001 mol) which was dissolved in hot ethanol (10 ml) and was mixed with a solution of the Schiff base (0.001mol) in acetonitrile (80 ml) and the resulting mixture was heated for 30 minutes. On standing overnight, the mixture yield crystalline complexes which were filtered off and dried in a desiccator over anhydrous silica gel, overnight. Yield: *ca.* 70%. Some analytical, physical and spectral data on the complexes can be found in Tables 1.

### **Physical measurements**

The analyses for carbon, hydrogen, nitrogen and sulphur were carried out using a LECO CHNS-932

instrument. The IR spectra in KBr pellets were recorded on a Perkin-Elmer FT IR 1750X spectrophotometer ( $4000\text{-}400\text{ cm}^{-1}$ ). Metal determinations were carried out using a Perkin-Elmer Plasma 1000 Emission Spectrometer. The molar conductance of  $10^{-3}\text{ M}$  solutions of the metal complexes in DMSO were measured at  $29^\circ\text{C}$  using a Jenway 4310 conductivity meter and a dip-type cell with a platinized electrode. Magnetic susceptibilities at room temperature were measured using a Sherwood Scientific MSB-AUTO magnetic susceptibility balance. The UV-VIS spectra were run on a Shimadzu UV-2501 PC/ Shimadzu UV-Vis-1650 recording Spectrophotometer ( $1000\text{-}200\text{ nm}$ ). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectrum of the Schiff base was recorded using a JOEL-JNM ECA 400 MHz spectrometer.

## Bioactivity

### Target microorganisms

Eight pathogenic microbials were used to test the biological potential of the complexes. They were Methicillin resistant *staphylococcus* (MRSA), *Bacillus subtilis* wild type (B29), *Pseudomonas aeruginosa* (60690), *Salmonella.Choleraesuis* (S.C) *Candida albicans* (C.A.), *Aspergillus ochraceous* (398) and *Saccaromyces ceciricae* (20341). The source of microbes and culture maintenance were as previously described [10].

### Qualitative antimicrobial assay

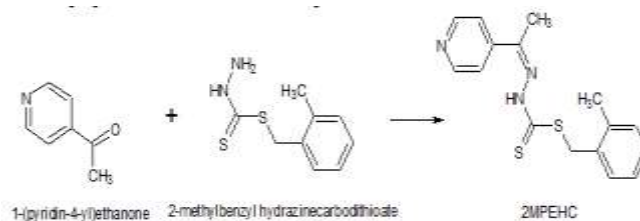
Antimicrobial activity of each sample was qualitatively determined by a modified disc diffusion method [11] as previously detailed [12]. A lawn of microorganisms was prepared by pipetting and evenly spreading inoculum ( $10^{-4}\text{ cm}^3$ , adjusted turbidometrically to  $10^5\text{ - }10^6\text{ cfu cm}^3$  (cfu: colony forming units) on to agar set in Petri dishes, using Nutrient agar (NA) for the bacteria and potato dextrose agar (PDA) for fungi. Whatman No. 1 filter paper discs of 6 mm diameter were impregnated with stock solution of the compound ( $100\text{ mg cm}^{-3}$ ) and dried under sterile conditions. The dried discs were then placed on the previously inoculated agar surface. The plates were inverted and incubated for 24h at  $30^\circ\text{C}$  for bacteria and  $37^\circ\text{C}$  for fungi. Antimicrobial activity was indicated by the presence of clear inhibition zones around the discs. Commercially available streptomycin (Sigma, USA) was used for the antibacterial control while nystatin (Sigma, USA) was used as the antifungal control.

## Cytotoxic assay

The MCF-7 (Human breast cancer cells with positive estrogen receptor) and MDA-MB-231 (Human breast cancer cells with negative estrogen receptor) cell lines were obtained from the National Cancer Institute, U.S.A. The cells were cultured in RPMI-1640 / DMEM (High glucose) (Sigma) medium supplemented with 10% fetal calf serum, Cytotoxicity was determined using the microtitration of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma, USA) as reported by Mosmann [17]. Controls that contained only cells were included for each sample. Cytotoxicity was expressed as  $\text{CD}_{50}$ , i.e. the concentration that reduced the absorbance of treated cells by 50% with reference to the control (untreated cells). Tamoxifen was used as the standard cytotoxin.

## Results and Discussion

Scheme 1 shows the condensation reaction to prepare the new ligand, while the physical and analytical data of the complexes are shown in Table 1. The analytical data agree well with the proposed formulations of the complexes.



Scheme 1: The synthesis equation of the new ligand

Table 1: physical properties and CHNS of the synthesized compounds

Compound	Colour	Melting point ( $^\circ\text{C}$ ) $\pm 1^\circ\text{C}$	% Found(calculated)				
			%C	%H	%N	%S	%M
LG2MPEHC $\text{C}_{12}\text{H}_{12}\text{N}_2\text{S}_2$	Orange	190	59.23 (60.92)	3.34 (3.43)	12.65 (13.32)	22.01 (20.33)	-
$\text{Ni}(\text{L})_2$	Green	136	56.05 (55.90)	3.02 (4.69)	11.96 (12.22)	18.87 (18.65)	8.52 (8.54)
$\text{Cd}(\text{L})_2$	Light Brown	210	52.02 (51.85)	4.32 (4.35)	11.33 (11.34)	17.05 (17.30)	14.89 (15.16)
$\text{Zn}(\text{L})_2$	Orange	200	56.02 (55.36)	4.36 (4.65)	11.96 (12.10)	18.25 (18.47)	9.33 (9.42)

### Infrared Spectroscopy (FTIR)

The IR spectral data for the Schiff bases and their metal(II) complexes are shown in Table 2. Four key functional group bands were selected to provide a comparative analysis, as these bands shift to higher or lower wavenumbers upon complexation with the metal(II) complexes<sup>(13)</sup>. The  $\nu(\text{C}=\text{N})$ ,  $\nu(\text{N}-\text{N})$ ,  $\nu(\text{CSS})$  and  $\nu(\text{NH})$  bands arise from the HNNS/ HNS Schiff base. The presence of the  $\nu(\text{C}=\text{N})$ ,  $\nu(\text{N}-\text{N})$  and  $\nu(\text{CSS})$  bands at  $1638\text{-}1586\text{ cm}^{-1}$ ,  $1040\text{-}1036\text{ cm}^{-1}$  and  $782\text{-}776\text{ cm}^{-1}$  respectively indicates that the coordination of the Schiff base to the central metal(II) ion is *via* the pyridyl nitrogen-if sterically unhindered, the azomethine nitrogen and the thiolate sulphur atom.

Table 2: Fourier Transform Infrared Spectroscopy (FT IR) data

Ligand/ Complex	$\nu(\text{C}=\text{N})$	$\nu(\text{N}-\text{N})$	$\nu(\text{CSS})$	N (NH)
L(2MPEHC)	1638 m	1038 s	776 m	3036 w
Ni(L) <sub>2</sub>	1614 m	1033 s	784 m	-
Cd(L) <sub>2</sub>	1584 m	1042 s	779 m	-
Zn(L) <sub>2</sub>	1609 m	1031 s	782 m	-

From the observation, the free benzyl hydrazinecarbodithioate ligand does not display band at  $\nu(\text{S}-\text{H})$  which are attributed to *ca*(S-H), but its display a very strong intensity at  $\nu$  ( $1048\text{ cm}^{-1}$ , bending) which represent as C=S which clearly indicates it remains in the thione form in the solid state. In the Schiff base, there is no peak assigned to C=S indicating that the Schiff bases appear in thiole form. There is peak at ( $1638\text{ cm}^{-1}$ ) which attribute to C=N, from this observation, it is concluded that the Schiff bases are formed via condensation reaction with condensation 1-(pyridin-4-yl)ethanone in which the primary amine reacted with the carbonyl group to form the new ligand. For complexes of nickel(II) cadmium(II) and zinc there is peaks assigned at ( $784\text{ cm}^{-1}$   $779\text{ cm}^{-1}$  and  $782\text{ cm}^{-1}$ ) which attribute to CSS which indicate the shifting of this band to higher frequency due to coordination of thiole sulphur with central metal ion. This only can happen if ligand in thiole form. It indicating that the ligand were undergoes deprotonated and acted as tridentate uni-negative chelating agents. C=N showed at peak ( $1638\text{ cm}^{-1}$ ) but as we can see that in the metal complexes, it is shift to the lower frequency to  $1584\text{-}1614\text{ cm}^{-1}$  this indicating that the metal bonded through azomethine nitrogen atom. A strong band at  $1038\text{ cm}^{-1}$  in the IR spectrum of the Schiff base is

assigned to the hydrazinic  $\nu(\text{NN})$  band. Its shift to lower wave numbers in spectra of all the metal complexes ( $1031\text{-}1042\text{ cm}^{-1}$ ) reflects reduction in the repulsion between the lone pairs of electrons on the nitrogen atoms and is certainly due to coordination *via* the azomethine nitrogen atom.<sup>[13-18]</sup> Figures 1, 2, 3 and 4 shows the IR-Spectra of the Schiff base and the Ni(II), Zn(II) and Cd(II) metal complexes respectively.

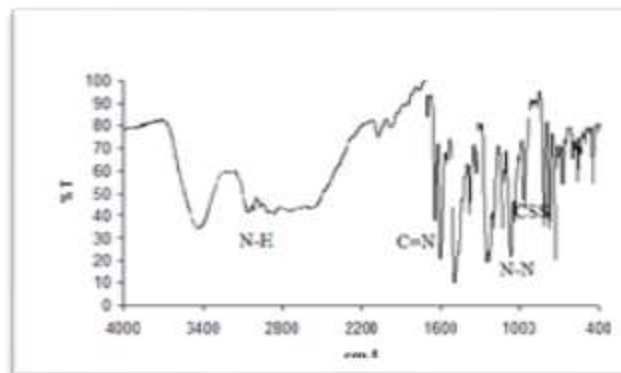


Figure 1: IR Spectrum of the new Schiff Base (2MPEHC)

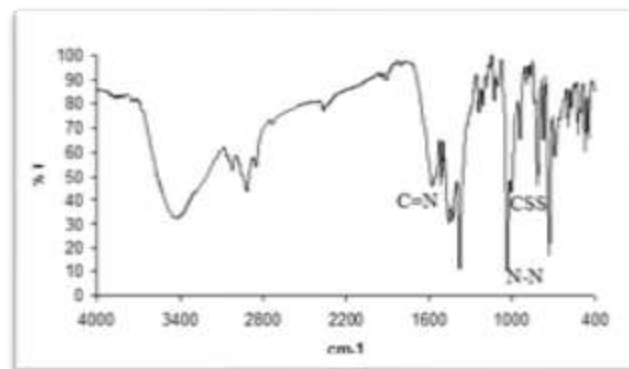


Figure 2: IR Spectrum of Ni(2MPEHC)<sub>2</sub>

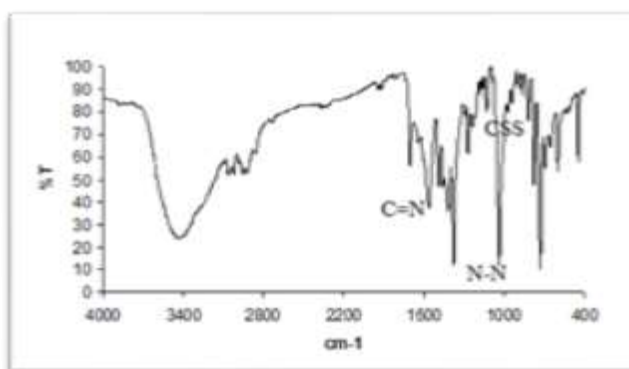


Figure 3: IR Spectrum of Zn (2MPEHC)<sub>2</sub>

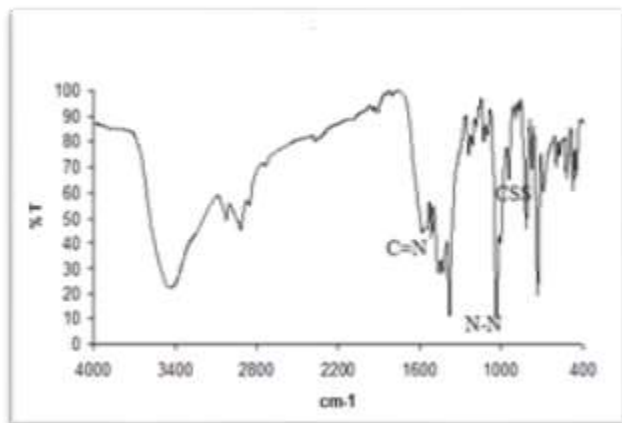


Figure 4: IR Spectrum of Cd(2MPEHC)2

### Molar conductivity, Magnetic Susceptibility and UV-Spectroscopy

Table 3 shows the molar conductance data, all the complexes were nonelectrolytes, representing that the ligand is coordinated to the metal ions as a uninegatively charged tridentate species. The molar conductance values of the complexes in DMSO fall in the range of 1.65 to 4.34  $\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$  indicating that they are essentially non-electrolytes in DMSO. Although some dissociation of the complexes seems to occur, the conductance values are much lower than that expected for a 1:1 electrolyte in this solvent [19]. Table 3 shows also the magnetic susceptibility data and as it is observed, all the complexes has diamagnetic natures. The coordinated complex of Ni(II) predictable to have square planar geometry, while it is suggested tetrahedral for Cd(II) and Zn(II) complexes [20].

Table 3: Molar conductivity, Magnetic

Complex	$\Lambda$ ( $\text{S cm}^2 \text{ mol}^{-1}$ )	$\mu_{\text{eff}}$ (BM)	$\lambda_{\text{max}}$ (Log $\epsilon$ ) (nm)
L	-	-	320 (2.90)
Ni(L) <sub>2</sub>	1.65	diamagnetic	310 (3.11), 370 (2.54), 440 (2.30)
Cd(L) <sub>2</sub>	4.34	diamagnetic	290 (3.34)
Zn(L) <sub>2</sub>	2.90	diamagnetic	355 (2.00)

The electronic spectra of the complexes in DMSO (Table 3) exhibits intra-ligands  $n \rightarrow \pi^*$  band at 320 nm. The presence of a  $S \rightarrow \text{Ni(II)}$  LMCT band at 440 nm in the spectra of some of the metal complex of Ni(II) is indicative of the coordination of the Schiff base to the M(II) ion *via* the thiolate sulphur atom. Both

the Cd(II) and Zn(II) complexes do not exhibit any d-d bands as they are  $d^{10}$  metal ions.

### NMR Analysis

The  $^{13}\text{C}$  NMR spectra of Schiff base showed a weak singlet at 198.72 ppm, which can be safely attributed to the thioamide carbon. The low intensity is due to the deshielding effect of two electronegative sulphur and one nitrogen atoms neighboring to the carbon atom. The electronegative elements produced a large downfield shift since it directly attached to the carbon atom. The C=N signal of the Schiff base occurred at *ca.* 164.72 ppm, indicative of the coordination of the azomethine bond which is formed when the particular ketone/aldehyde reacts with the dithiocarbazate. The -S-CH<sub>2</sub> peak occurred comparatively upfield, around 36.25 ppm, maybe due to the electropositive effects of the neighbouring methylbenzyl ring, which is electron-donating in nature. The aromatic carbons occurred as group of peaks very near each other around 126.16–154.15 ppm, which was around as expected of carbons in an aromatic ring. CH<sub>3</sub> group could be noticed at 18.92 and 13.07 ppm, it appears due to the electron delocalization of the benzene ring. Table 4 shows the main peaks in  $^{13}\text{C}$  spectra of the Schiff base.

Table 4:  $^{13}\text{C}$  NMR Data of the Schiff base

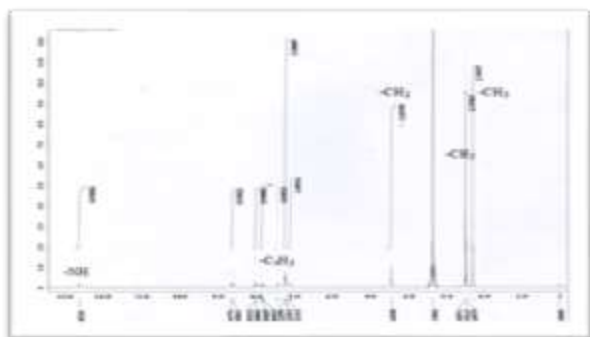
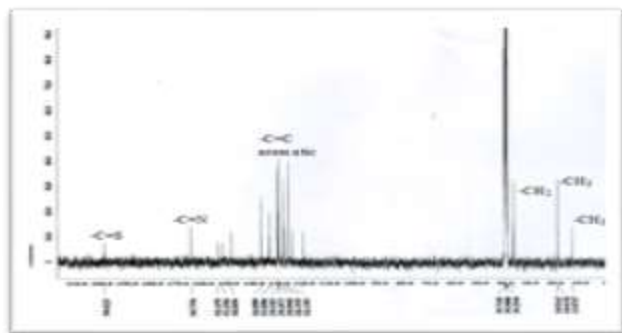
$^{13}\text{C}$ NMR ( $\delta$ , ppm)	L
C=S	198.82
C=N	164.72
-Ar-H	126.16-154.15
-S-CH <sub>2</sub>	36.25
-CH <sub>3</sub>	18.92, 13.07

DMSO was used as a solvent to the  $^1\text{H}$  NMR spectra of the Schiff base, the spectra indicates the existence of phenyl rings protons which attach to the phosphorus appeared as multiplet signals around region 7.21 – 8.62 ppm roughly similar to the chemical shift of phenyl rings protons moreover, the -NH proton signal located at 12.62 ppm (1H). A singlet peak come into view on 4.44 ppm representing the attendance of S-CH<sub>2</sub> group confirming the structure of Schiff base that proposed, two more singlet appear in 2.49 ppm and 2.51 ppm which attributed to -CH<sub>3</sub>. [21]

Table 2:  $^1\text{H}$  NMR Data of S2MBDTC and its Schiff bases

$^1\text{H}$ NMR ( $\delta$ , ppm)	L
$-\text{NH}_2$	-
$-\text{NH}$	12.62 (singlet, 1H)
$-\text{Ar-H}$	7.21-8.62 (multiplet, 8H)
$-\text{S-CH}_2$	4.44 (singlet, 2H)
$-\text{CH}_3$	2.49, 2.51 (singlet, 6H)

Figures 5 and 6 shows the  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR for the new Schiff base

Figure 5:  $^1\text{H}$  NMR Spectrum of the Schiff base (2MPEHC)Figure 6:  $^{13}\text{C}$  NMR Spectrum of the Schiff base (2MPEHC)

## Biological activities

### Antimicrobial activities

The antimicrobial activities were measured from the diameter of clear inhibition zones. It has been observed that the Schiff base, cadmium, zinc and nickel complexes were active against the tested bacteria only and doesn't shows any activities against the fungal

strains (inhibitory zones  $> 15$  mm). Table 4 shows all the measurement data <sup>[10]</sup>.

Table 4: Qualitative Antimicrobial and Antifungal screening result of the Schiff base and its Transition Metal Complexes

Complex	Inhibition diameters (mm) <sup>a</sup>				Fungal Strains		
	Bacterial Strains				C.Alb	A.Och	S.Cer
	MRSA	P.Aer	S.Cho	B.Sub			
L	-	-	-	11	-	-	-
Ni(L) <sub>2</sub>	8	-	-	13	-	-	-
Cd(L) <sub>2</sub>	20	-	-	25	-	-	-
Zn(L) <sub>2</sub>	20	20	20	20	-	-	-
Streptomycin	20	20	24	23	-	-	-
Nystatin	-	-	-	-	23	24	28

Code: MRSA – Methicillin resistant *Staphylococcus Aureus*, P.aer – *Pseudomonas aeruginosa*, S.cho – *Salmonella cholerasuis*, B.Sub – *Bacillus subtilis*- wild type, C.alb – *Candida albicans*, A.och – *Aspergillus ochraceous*, S.cer – *Saccharomyces cerevisiae*

<sup>a</sup> Inhibition diameter  $> 15$  mm is strongly active; - indicates 'not active'

### Cytotoxic activities

The Schiff base and complexes were evaluated against MDA-MB-231 (Human breast carcinoma cells with negative estrogens receptor) and MCF-7 (Human breast carcinoma cells with positive estrogens receptor). Measurement for cytotoxicity was in  $\text{IC}_{50}$ , where  $\text{IC}_{50}$  refers to cytotoxic dose at 50% i.e. the concentration to reduce growth of cancer cells by 50% <sup>[10]</sup>. As it is clear from Table 7, Cd(II) and Zn(II) complexes shows high activity only against MCF-7 <sup>[22]</sup>.

Table 7: Cytotoxic Data of the Schiff base and its Transition Metal complexes

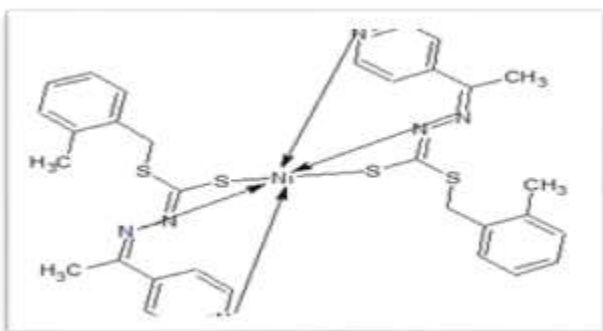
Complex	$\text{IC}_{50}$ ( $\mu\text{g/ml}$ )	
	MCF-7	MDA-MB-231
L	Inactive	Inactive
Ni(L) <sub>2</sub>	Inactive	Inactive
Cd(L) <sub>2</sub>	0.7	Inactive
Zn(L) <sub>2</sub>	1.3	Inactive
Tamoxifen	5.0	5.5

<sup>c</sup>  $\text{IC}_{50} < 5.0 \mu\text{g cm}^{-3}$ - strongly active,  $\text{IC}_{50} 5.0 < 10.0 \mu\text{g cm}^{-3}$ - moderately active,  $\text{IC}_{50} 10.0 < 25.0 \mu\text{g cm}^{-3}$ - weakly active,  $\text{IC}_{50} > 25.0 \mu\text{g cm}^{-3}$ - not active.  $\text{IC}_{50}$  ( $\mu\text{g cm}^{-3}$ ) = Cytotoxic dose at 50% i.e. the

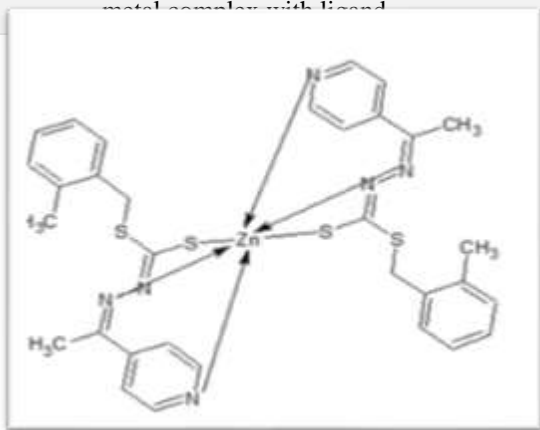
concentration to reduce growth of cancer cells by 50%. MCF-7= Human Breast Carcinoma Cells with Positive Estrogen Receptor, MDA-MB-231 = Human Breast Carcinoma Cells with Negative Estrogen Receptor

### Conclusions

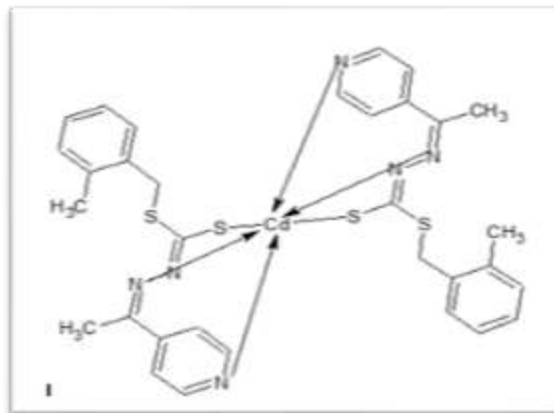
New nickel(II) cadmium(II) and zinc(II) complexes of 2-methylbenzyl(2Z)-2-[1-(pyridin-4-yl)ethylidene]hydrazine carbodithioate (L) Schiff base were synthesized. The Schiff base coordinates to the central metal ions as a uninegatively charged bidentate *N,S* ligand through the azomethine nitrogen and thiolate sulfur moiety. The bioactivity of the complexes indicated that the metal complexes were selectively active against the MCF-7 cell line as compared to the MDA-MB-231 cell line. The general order of cytotoxic activity of the complexes against the MCF-7 breast cancer cell lines  $Cd(L)_2 > Zn(L)_2$ , while Ni(II) metal complex is not active. The complexes were also more antibacterial than antifungal in nature. Schemes 2, 3 and 4 shows the expected coordination of the metal complexes with the ligand



Scheme 2: The expected coordination of Ni (II) metal complex with ligand



Scheme 3: The expected coordination of Zn (II) metal complex with ligand



Scheme 4: The expected coordination of Cd(II) metal complex with ligand

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