Preparation, Identification and Biological Study of new Cytoxically Active Schiff Base Derived from 1-(pyridin-3-yl)ethanone and its metal complexes

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Abstract

A new ligand 4-methylbenzyl (2*E*)-2-[1-(pyridin-3-yl) ethylidene] hydrazinecarbodithioate (PE4MBHC) with nitrogen sulphur donor sequence was prepared from the reaction between 1-(pyridin-3-yl)ethanone (PE) and 4-methylbenzyl hydrazinecarbodithioate (4MBHC). Complexes of this ligand with Cu(II), Ni(II) and Zn(II) were synthesized and characterized using elemental analysis and various physicochemical techniques. The metal complexes were six coordinated and all the new compounds were tested against different bacteria and fungi and also against two breast cancer cells. All metal complexes showed moderate activity against microbes while copper and nickel shows strong activity against MCF7 and MDAMB231 respectively.

Introduction

Schiff bases has been widely used as ligands due to its coordination with metal ions forming coordination compounds, these complexes have been of great interest since 70th of the last century. It was observed that nitrogen-sulphur ligands and their metal complexes shows importance in biological system and also a small changes in their structures properties such as change the substituents and their chelating to different central metal ion will cause a great changes in their bioactivity and cytotoxicity [1-8] .

A new Schiff base, (2*E*)-2-[1-(pyridin-3-yl)ethylidene]hydrazinecarbodithioate (PE4MBHC) has been prepared by reacting 1-(pyridin-3-yl)ethanone (PE) and 4-methylbenzyl hydrazinecarbodithioate (4MBHC). The new ligand was reacted with the acetate salts of Ni(II), Cu(II) and Zn(II) to yield coordination metal complexes.

Experimental

Physical Measurements and Elemental Analyses

Melting point was determined using electrothermal digital melting point apparatus (Barnstead Electrothermal 9100), Carbon, hydrogen, nitrogen and sulfur analyses were carried out using a Leco CHNS-932 analyzer. The IR spectra were recorded in the range of 400–4000 cm^{-1} with KBr pellets on either a Perkin Elmer 1650X or 1750X infrared spectrophotometer. Magnetic susceptibility was measured with a Sherwood Auto MSB at 298 K. All susceptibilities were corrected for the diamagnetic contribution using Pascal's constant. The mass spectrum was recorded on the Shimadzu GCMS QP5050A mass spectrometer by using the technique of direct insertion (DI-MS). The molar conductance of a 10^{-3} M solution of each metal complex in DMSO was measured at 29 °C using a Jenway 4310 conductivity meter and a dip-type cell with platinized electrode. The UV–Vis spectra were recorded on a Shimadzu UV-1650 PC spectrophotometer (1000–200 nm). 1H NMR spectra were recorded on a NMR JNM ECA400 MHz NMR spectrometer. Metal determinations were carried out using a Perkin Elmer Plasma 2000 DV Optical Emission Spectrometer. All the above used instruments measurements were done at Chemistry Departement, University Putra Malaysia unless otherwise will be mentioned.

Preparation of 4-methylbenzyl hydrazinecarbodithioate (4MBHC).

11.4 gm (0.2 mol) of potassium hydroxide was dissolved in 70 ml of 95% ethanol. To this solution, 10 ml (0.2 mol) of hydrazine hydrate was added; 12 ml (0.2 mol) of carbon disulfide was added dropwise with constant stirring over a period of one hour. The light-brown oil layer was dissolved in 50 ml of 40% cold ethanol and kept in an ice-bath. 23 ml (0.2 mol) of benzyl chloride was added dropwise with vigorous stirring of the mixture. The off-white product, (SBDTC), which formed was filtered and washed with cold ethanol. The product was filtered and left to dry overnight in a desiccator over anhydrous silica gel. (Yield 79%, compound m.p 160 °C) [(9)] .

Preparation of (2*E*)-2-[1-(pyridin-3-yl)ethylidene]hydrazinecarbodithioate (PE4MBHC)

0.01 mol of 4MBHC was dissolved in hot acetonitrile (150ml). This was added to an equimolar amount of 1-(pyridin-3-yl)ethanone (PE) in ethanol (10 ml). The mixture was heated for 30 minutes and then allowed to stand for a few hours or placed in the refrigerator. The yellow crystals that formed were filtered, washed with cold ethanol and recrystallised from acetonitrile. Yields were 85%.

Preparation of Metal Complexes

M [acetate].nH_2O [M = Cu(II), Ni(II), and Zn(II)] (0.001 mol) dissolved in hot 95% ethanol (25 ml) was mixed with a solution of (2*E*)-2-[1-(pyridin-3-yl)ethylidene]hydrazinecarbodithioate (PE4MBHC) (0.002mol) in acetonitrile/ethanol (50 ml) and the resulting mixture was heated for around 30 minutes. On standing overnight, the crystalline complexes which were filtered, washed with cold ethanol and dried in a desiccator over anhydrous silica gel. Yields: 65-78%.

Anti-microbial activity

Eight pathogenic microbes were used to test the biological activity of the complexes: 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 ceciricaee (20341) [10]. Antimicrobial activity of each sample was qualitatively determined using diffusion method [11-12] . A lawn of microorganisms was prepared by pipetting and evenly spreading inoculum (10^{-4} cm^3, adjusted turbidometrically to 10^5–10^6 cfu cm^{-3} (cfu: colony forming units) onto agar set in Petri dishes, using nutrient agar (NA) for the bacteria and potato dextrose agar (PDA) for fungi. Whatman No. 1 filter paper disks of 6 mm diameter were impregnated with stock solution of the complex (100 mg cm^{-3}) and dried under sterile conditions. The dried disks were then placed on the previously inoculated agar surface. The plates were inverted and incubated for 24 h at 30 °C for bacteria and 37 °C for fungi. Antimicrobial activity was indicated by the presence of clear inhibition zones around the disks. Commercially available streptomycin (Sigma, USA) was used for the antibacterial control, while nystatin (Sigma, USA) was used as the antifungal control. Complexes that showed positive anti-microbial inhibition with the disk diffusion assay (diameter >15 mm) were subjected to the broth dilution method for the quantitative measurement of microbiostatic (inhibitory) activity as described by Hufford and Clark [13]. The lowest concentration that completely inhibited visible microbial growth was recorded as the minimum inhibitory concentration (MIC, µg, cm^{-3}). Streptomycin and nystatin were used as controls for bacteria and fungi, respectively.

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, USA. 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 [10]. Controls that contained only cells were included for each sample. Cytotoxicity was expressed as 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.

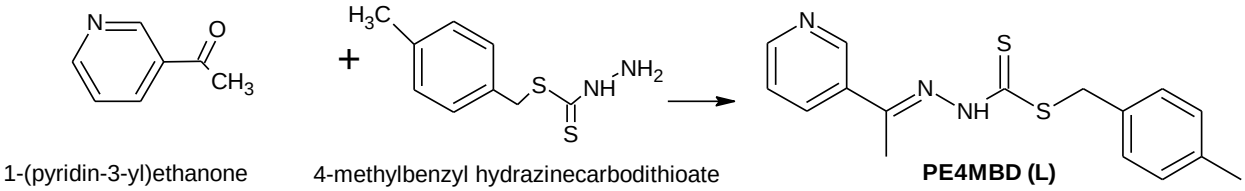
Results and discussion

The physical and analytical data for the complexes are shown in Table 1. The analytical data agree well with the proposed formulations of the complexes. The complexes are soluble in most organic solvents and appear stable in air.

Table 1: Physical properties and CHNS of the synthesized compounds

	%C	**%H**	**% N**	**%S**	**%M**		
4MBHC	52.05 (50.91)	5.92 (5.70)	13.92 (13.19)	32.58 (30.20)	-	White	**161**
L(PE4MBHC) $C_{16}H_{17}N_3S_2$	58.89 (60.92)	5.14 (5.43)	12.74 (13.32)	22.65 (20.33)	-	Light Yellow	**176**
$Cu(L)_2$	52.62 (55.51)	4.47 (4.66)	11.38 (12.14)	20.09 (18.52)	9.04 (9.18)	Green	**201**
$Ni(L)_2$	57.89 (55.90)	4.53 (4.69)	13.15 (12.22)	20.58 (18.65)	8.86 (8.54)	Brown	**187**
$Zn(L)_2$	**53.25 (55.36)**	**4.43 (4.65)**	**11.67 (12.10)**	**21.16 (18.47)**	**9.17 (9.42)**	**Light Yellow**	**208**

Scheme 1 shows the preparation equation of the new ligand from its raw material.



1-(pyridin-3-yl)ethanone 4-methylbenzyl hydrazinecarbodithioate **PE4MBD (L)**

Scheme 1: Synthesis equation of the new Schiff base

Magnetic properties and molar conductivity

The value of (μeff.) that have been measured for all the metal complex it was found (2.13 B.M) for Cu(II) and diamagnetic for both Ni(II) and Zn(II)

complexes respectively, Table 2.. These values are in the range of mononuclear octahedral geometry for all the metal complexes [(1)] .

The chelates were dissolved in DMSO and molar conductance 10^{-3} M of solution at room temperature was measured. The molar conductance values of the complexes fall in the range 4.17 to 6.94 indicating that these chelates are non-electrolytes [(14, 15)] .

Table 2: Magnetic susceptibility, molar conductivity and electronic spectra measurements of the metal complexes

Complex	Λ (S cm^2 mol^{-1})	μ_{eff} (BM) at R.T	λ_{max} (Log ε) (nm)
PE4MBD(L)	-	-	295 (2.99), 342 (3.13)
$Cu(L)_2$	4.87	2.13	317 (2.98), 386 (2.75), 641.32 (1.42)
$Ni(L)_2$	6.94	diamagnetic	277.50 (2.35), 447 (2.19)
$Zn(L)_2$	4.17	diamagnetic	295 (3.35)

Electronic Spectra

The electronic spectra data of the ligands and their complexes were recorded in dry DMSO. The various bands observed were assigned to interligand and charge transfer of π-π* and n-π* transitions according to their energies and intensities. Electronic spectral data of Schiff bases and its metal complexes are shown in Table 2. The spectra of the Schiff bases exhibit bands at 295 nm and 342 nm. The transitions were attribute to the carbonyl, thione and azomethine groups respectively [(16)] . The electronic spectra of copper complexes exhibit a *d–d* band at ca. 641 nm and intra-ligand bands at 317 nm and 386 nm respectively [(17)] . The appearance of a ligand to metal charge transfer (LMCT) band at 447 nm in the electronic spectra of the nickel(II) complexes is a strong evidence that the nickel(II) ion is coordinated to the Schiff base via the sulphur atom [(18)] . The electronic spectra of the zinc complex shows only an intraligand band at 295 nm [(19)] .

IR spectra

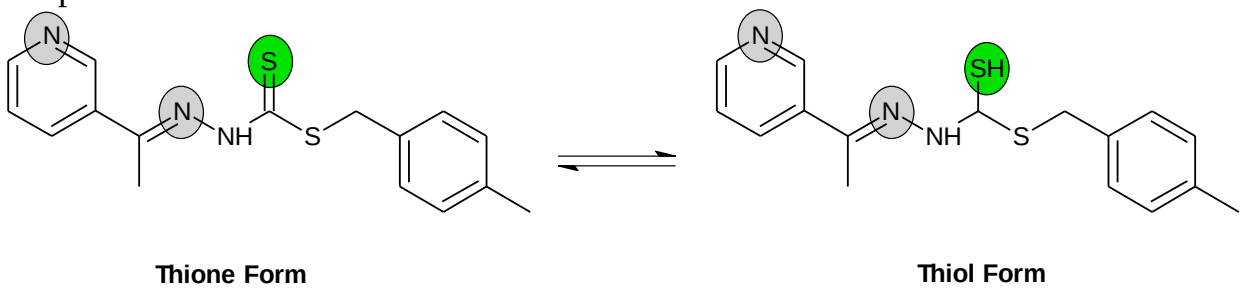
The infrared spectra of the reported compounds were recorded in the range 4000 –400 cm^{-1}. The assignment of IR bands of the synthesized compounds has been determined by a comparison with IR spectra of the precursors[(20)]. The most important infrared spectral bands that provide conclusive structural evidence for the coordination of the ligands to the central metal ions are given in Table 3. 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. The ν(C=N),

ν(N-N), ν(CSS) and ν(NH) bands arise from the HNNS/ HNS Schiff base. The presence of the ν(C=N), ν(N-N) and ν(CSS) bands at 1560-1614 cm^{-1}, 1017-1024 cm^{-1} and 929-945 cm^{-1} respectively indicates that the coordination of the Schiff base to the central metal(II) ion is *via* the pyridyl nitrogen, the azomethine nitrogen and the thiolate sulpur atom.

Table 3: IR Spectral data of the Schiff bases and their Transition Metal Complexes

4MBHC	**1621**	**1059**	**981**	**3389**
L	**1607**	**1021**	**837**	**3031**
$Cu(L)_2$	**1614**	**1020**	**936**	**-**
$Ni(L)_2$	**1560**	**1024**	**945**	**-**
$Zn(L)_2$	**1588**	**1017**	**929**	**-**

There was no ν(SH) observed in the IR of the Schiff bases at 2600 cm^{-1}, but the ν(NH) band was observed at 3031 cm^{-1} indicating that, in the solid state, it remains as the thioketo tautomer as appear in Scheme 2. The ν(NH) band is absent in the spectra of the M(II) complexes lending further support to the suggestion that the Schiff bases deprotonate during coordination to the M(II) complexes [21].



Thione Form **Thiol Form**

Scheme 2: Thione thiol tautomers

NMR Analysis

^{1}H NMR Spectral Analysis

^{1}H NMR spectra of the Schiff base was taken using DMSO as a solvent, Table 4 shows the main peaks of the spectra which indicates the existence of phenyl rings protons as multiplet signals at region 7.06 – 8.90 ppm roughly similar to the chemical shift of phenyl rings protons, while the –NH proton signal appear at 9.30 ppm (1H). two more singlet appear in 2.49 ppm and 2.51 ppm which attributed to $-CH_3$. A singlet peak on 4.36 ppm representing the attendance of S-

CH_2 group confirming the structure of Schiff base that proposed [22].

Table 4 : ^{1}H NMR Data of 4MBHC and its Schiff bases

^{1}H NMR (δ,ppm)	4MBHC	L
-NH_2	4.36 (singlet, 2H)	-
-NH	9.30 (singlet, 1H)	12.50 (singlet, 1H)
-Ar-H	7.05 – 7.19 (multiplet, 5H)	7.06-8.90 (multiplet, 9H)
-S-CH_2	4.44 (singlet, 2H)	4.36 (singlet, 4H)
-CH_3	2.28 (singlet, 3H)	2.21, 2.45 (singlet, 9H)

^{13}C NMR Spectral Analysis

The ^{13}C NMR spectra of Schiff base showed a singlet at 199.05 ppm, which can be safely attributed to the thioamide carbon C=S while the C=N signal of the Schiff base occurred at 165.13 ppm, indicative of the coordination of the azomethine bond. The –S-CH_2 peak occurred at 37.50 ppm, due to the electropositive effects of the neighbouring methylbenzyl ring. The aromatic carbons occurred as group of peaks at 130.06–138.06 ppm, which was around as expected of carbons in an aromatic ring. CH_3 groups were noticed at 20.94 ppm and 14.78 ppm, it appears due to the electron delocalization of the benzene ring. Table 5 shows the main peaks in ^{13}C spectra of the Schiff base [23] .

Table 5: ^{13}C NMR Data of 4MBHC and its Schiff bases

^{13}C NMR (δ,ppm)	**4MBHC**	**L**
C=S	**-**	**199.05**
C=N	**-**	**165.13**
-Ar-H	**130.06-138.06**	**123.95-150.73**
-S-CH_2	**39.50**	**37.50**
-CH_3	**21.15**	**20.94, 14.78**

Antimicrobial results

In vitro antibacterial and antifungal activity of the ligands and their corresponding metal complexes was tested against certain bacteria and fungus as shown in Table 6. The results show that all compounds exhibit a moderate

antimicrobial activity.

Table 6: Qualitative Antimicrobial and Antifungal screening result of the Schiff base and its Transition Metal Complexes

Complex	Inhibition diameters (mm)						
		Bacterial	Strains		Fungal Strains		
	MRSA	P.Aer	S.Cho	B.Sub	C.Alb	A.Och	S.Cer
L	13	-	-	-	-	-	-
$Cu(L)_2$	13	-	13	13	-	-	-
$Ni(L)_2$	11		12	-	-	-	-
$Zn(L)_2$	13	-	12	13	-	-	-
Streptomycin	21	22	24	23	-	-	-
Nystatin	-	-	-	-	22	22	25

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*

[a] Inhibition diameter > 15 mm is strongly active; - indicates 'not active'

Cytotoxic activities

The Schiff base and the metal 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 IC_{50} [(10)]. As it is clear from Table 7, Ni(II) and Cu(II) complexes shows high activity only against MDAMB-231 and MCF-7 respectively [(24)].

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

Complex	IC_{50} (µg/ml)	
	MCF-7	MDA-MB-231
L	5.32	Inactive
$Cu(L)_2$	0.38	Inactive
$Ni(L)_2$	2.12	0.28
$Zn(L)_2$	Inactive	1.8
Tamoxifen	6.0	6.5

IC50 < 5.0 µg cm^{-3}- strongly active, IC50 5.0 < 10.0 µg cm^{-3}- moderately active, IC50 10.0 < 25.0 µg cm^{-3}- weakly active, IC50 > 25.0 µg cm^{-3}- not active.

IC50 (µg cm^{-3}) = Cytotoxic dose at 50% i.e.

MCF-7= Human Breast Carcinoma Cells with Positive Estrogen Receptor,

MDA-MB-231 = Human Breast Carcinoma Cells with Negative Estrogen Receptor

Molecular Modeling and Analysis

In view of the six-coordination of the present complexes, $[M(L)_2]$, the molecular modeling of the compound as a representation, is based on its octahedral structure.

Conclusions

We report here the synthesis and characterization of new ligand and its Cu(II), Ni(II) and Zn(II) complexes with the Schiff base derived from 1-(pyridin-3-yl)ethanone (PE) and 4-methylbenzyl hydrazinecarbodithioate (4MBHC). The synthesis were conducted in conditions allowing the tridentate ligand and the stability of the metal (II) complexes. The synthesized compounds characterized by various physicochemical techniques like elemental analysis, IR, UV, 1H and 13C NMR as well as by conductance measurements. The correlation of the experimental data allows assigning a six-coordinated to all the reported complex compounds. The free ligands and their metal complexes were screened against various fungi and bacteria to access their potential as antimicrobial agents and it was found that all the new compounds shows a moderate bioactivity while the screening against breast cancer cell lines (MCF-7 and MDAMB-231) shows a very good activity especially Ni(II) and Cu(II) complexes.

References

1. Shamkhy, E.T and Al-Karkhi, I.H.T (2012). Synthesis, Characterization and Spectroscopic Studies of A Novel 2-[(E)-[(2,4- dichlorophenyl)imino]methyl]phenol Schiff Base and Its Metal Complexes. E-Journal of Chemistry. **9(3)**, 1543-1549.
2. Tarafder, M.T.H., Jalil, M.M.A., Bose, R.N. and Akbar M.A. 1981. Metal complexes of some Schiff bases derived from S-benzyldithiocarbazate. *Journal of Inorganic and Nuclear Chemistry* 43(12): 3151 – 3157.
3. Ali, M.A. and Tarafder, M.T.H. 1977. Metal complexes of sulphur and nitrogen-containing ligands: Complexes of S-benzyldithiocarbazate and a Schiff base formed by its condensation with pyridine-2-carboxaldehyde. *Journal of Inorganic and Nuclear Chemistry*. 39: 1785-1791, and references therein.
4. Ali, M.A.,Majumder., S.M., Butcher., R.J., Jasinski., J.P. and Jasinski, J.M. 1997. The preparation and characterization of bis-chelated nickel(ll) complexes of the 6-methylpyridine-2-carboxaldehyde Schiff bases of S-alkyldithiocarbazates and the X-ray crystal structure of the bis {S-methyl-β-N- (6-methylpyrid-2-yl)-methylenedithiocarbazato} nickel(ll) complex. *Polyhedron* 16(16): 2749-2754, and references therein.
5. Ali, M.A., Mirza, A.H., Fatriah, H.B., Malai, H., Hamid, S.A. and Paul V. B. 2006. Synthesis, characterization and X-ray crystallographic structural study of copper(II) and nickel(II) complexes of the 2-quinoline carboxaldehyde Schiff base of S-methyldithiocarbazate (Hqaldsme). *Polyhedron* 25: 3245–3252.
6. Ali, M.A., Hj. A.B., Mirza, A.H., Smith, S.J., Gahan, L.R. and Bernhardt, P.V. 2008. Preparation, spectroscopic characterization and X-ray crystal and molecular structures of nickel(II), copper(II) and zinc(II) complexes of the Schiff base formed from isatin and *S*-methyldithiocarbazate (Hisa-

sme). *Polyhedron* 27(1): 71-79.
7. Sadaf, K., Nami, S.A.A., Siddiqi, K.S., Eram, H. and Imrana N. 2009. Synthesis and characterization of transition metal 2,6-pyridinedicarboxylic acid derivatives, interactions of Cu(II) and Ni(II) complexes with DNA in vitro. *Spectrochimica Acta* 72: 421–428.
8. Singh, J., Hundal, G., Corbella, M. and Gupta, R. 2007. Synthesis, Characterization and Structures of Copper(II) Complexes with Amide-Based Ligands: Unusual Formation of a Linear Trimer and a Zig-zag Chain and Their Contrast Magnetic Behaviour. *Polyhedron* 26: 3893-3903.
9. Thahira B. 2008. Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy.
10. Mosmann, T. 1983. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to proliferation and Cytotoxicity Assays. *Journal of Immunological Methods* 65: 55-63.
11. Bauer, A.W. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45: 493-496.
12. Zhang, Y., Wang Yebin and Yu Heng (1995), New Metal Complexes Derived from 1-Ferrocenecarbonyl-4-phenyl-3-thiosemicarbazide with Some Transition Metal Ions *Cryst. Res. Technol*., **30**: 831.
13. Hufford CD, Clark AM (1988) In: Ur-Rahman A (ed) Discovery and Development in New Drugs for Systemic Opportunistic Infections. Studies in Natural Product Chemistry, 2, Elsevier, Amsterdam, p 421
14. Singh, P.K. and Kumar, D.N. (2006). Spectral studies on cobalt(II), nickel(II) and copper(II) complexes of naphthaldehyde substituted aroylhydrazones. *Spectrochimica Acta Part A* 64: 853-858.
15. Ali, M.A., and Tarafder, M.T.H. (1977). Metal complexes of sulphur and nitrogen-containing ligands: Complexes of *S*-benzyldithiocarbazate and Schiff base formed by its condensation with pyridine-2-carboxaldehyde. *J. Inorg. Nucl. Chem* 39: 1785-1791.
16. Murukan, B. and Mohanan, K. (2006). Synthesis, characterization, electrochemical properties and antibacterial activity of some transiton metal complexes with [(2-hydroxy-1-naphthaldehyde)-3-isatin]bishydrazone. *Transition Met. Chem.* 31: 441-446.
17. West, D.X., Liberta, A.E., Padhye, S.B., Chikate, R.C., Sonawane, P.B., Kumbhar, A.S. and Yerande, R.G. (1993). Thiosemicarbazone complexes of copper(II): structural and biological studies. *Coord. Chem. Rev.* 123: 49-71.
18. El-Sayed, L., Iskander, M.F., El-Toukhy, A. and Zayan, S.E. (1972). Coordination compounds of hydrazine derivatives with transition metals. V. Square planar nickel(II) complexes with hydrazine-S-methyl dithiocarboxylate schiff bases. *Inorg. Chim. Acta* 6: 663-668.
19. Shamkhy, E.T and Al-Karkhi, I.H.T. (2011). Preparation of New Schiff Base Derived from Cyclohexylamine with Piperonaldehyde and its Cu+2, Co+2 and Rh+3 Metal Complexes Oriental Journal of Chemistry. Vol. 27(4): 1403-1408.
20. Carlin, R. L. (1965). Transition Metal Chemistry, Second ed., Marcel Decker, New York.
21. Hossain, M.E., M.N Alam, M.A Ali, M.Nazimuddin, F.E Smith and R.C Hynes (1996). The synthesis, characterisation and bioactivities of some Copper(II) complexes of the 2-acetylpyridine Schiff bases of S-Methyl- and S-benzyldithiocarbazate, and the X-ray Crystal Structure of the Nitrato(S-Benzyl- β -N-(2-Acetylpyridyl) methylenedithiocarbazato)Copper(II) Complex, *Polyhedron*, **15,5-6**: 973-980.
22. Anjaneyulu, Y. and . Prabhakara., R.R. 1986 Preparation, Characterization and Antimicrobial Activity Studies on Some Ternary Complexes of Cu(II) with Acetylacetone and Various Salicylic Acids. Synth. React. Inorg. Met.-Org. Nano-Met. Chem. 16(2): 257 – 272.
23. Talath.S and A.K. Gadad (2006) Synthesis, antibacterial and antitubercular activities of some 7-[4-(5-amino-[1,3,4] thiadiazole-2-sulfonyl)-piperazin-1-yl] fluoroquinolonic derivatives, *Eur.J.Med Chem*, 41(8): 918 – 924.
24. Jessica, E.H., Jason R.B., Emily, K.R., Kayleigh, T., Breunig, J.S., Elizabeth, S. H., Kevin,

T.V. and Edward H.H. 2008. Live-Cell Analysis of Mitotic Spindle Formation in Taxol-Treated Cells. Cell Mot. Cyt. 65(8): 595–613.