

Original Research Article

SYNTHESIS, SPECTRAL CHARACTERIZATION OF SCHIFF BASE TRANSITION METAL COMPLEXES: ACHE INHIBITOR, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY STUDIES

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ABSTRACT

In the present study, we synthesized Novel ligand and their metal complexes with 8-((2-(2,4-dinitrophenyl)hydrazono)methyl)-7-hydroxy-4-methyl-2H-chromen-2-one ligand. The colored complexes were prepared by the addition of chloride salts of Manganese(II), Cobalt(II), Nickel(II) and Copper(II) to a solution of ligand. In conclusion, the structures of the obtained complexes were characterized by FT-IR, elemental analysis, UV-spectral studies, conductmetric and magnetic susceptibility measurements. The synthesized metal complexes were investigated for biological activities. Enzymatic inhibition activity has been done by using acetylcholinesterase (AChE) enzyme, antioxidant activity by DPPH assay and also the antimicrobial studies. All tested metal complexes and ligand reveal effective biological activities.

Key words; Schiff bases, Metal complexes, Enzymatic activity, DPPH Assay, Antimicrobial activity.

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INTRODUCTION

In the recent years it has been the trend to synthesize the ligand molecules having definite frame work of donor atoms and co-ordinate them with different metal ions such of the complexes have been further explored for their catalytic and biological activities [1, 2]. For the last few years much attention has been casted on polyfunctional ligands, which can encapsulate the metal ions. Multi-metal complexes have acquired a position of significance in the area of bio-inorganic chemistry. Many enzymes and proteins involving multimetal systems have also been reported [3, 4]. Coumarin is structurally the least Complex member of large class of compounds known as benzopyrones. Coumarin constitute an important class of compounds with several type of pharmacological agents possessing anticancer, anti-HIV [5], anticoagulant, antithrombotic, antimicrobial [6], antiallergic, anti-inflammatory and spasmolytic [7]. It has been found that the

binding of a metal to the coumarin moiety retains or even enhances its biological activity [8]. The enzyme acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine to acetate and choline at the cholinergic synapses [9], terminating nerve impulse transmission. It is known that AChE is effectively inhibited by organophosphate and carbamate pesticides, and also by metals [10].

Since the late 1980 a number of in-vitro and in-vivo studies have investigated the possible use of coumarins in the treatment of cancer [11]. The in-vitro effects of coumarins on the growth of cell carcinoma that derived cell lines show that coumarins and 7-hydroxy coumarin were potent cytotoxic and cytostatic agents [12]. The synthesis and biological importance of Co(II), Ni(II) and Cu(II) metal complexes of triazole Schiff bases have effective biological important [13] and also the lanthanide complexes of 4-methyl-7-hydroxycoumarin shows effective pharmacological activity [14]. The newly synthesized compounds were assayed for acute intraperitoneal and per oral toxicity, influence on blood clotting time and for spasmolytic activity [15].

A survey of the literature aroused our interest in synthesizing and elucidating the structures of transition metal complexes with following new coumarin ONO donor Schiff base and also their biological importance in various fields.

MATERIALS AND METHODS

The chemicals used for synthesizing the precursors, Schiff base and metal complexes were of reagent grade. Organic solvents used included absolute ethylalcohol, diethylether, formamide (DMF). Reagent grade organic solvents were purified and dried by recommended procedures [16]. Hydrochloric and nitric acids (E-Merck) were used. De-ionized water collected from all glass equipments were usually used in all preparations and also for biological applications.

Carbon, hydrogen, nitrogen and oxygen were estimated by using Elemental Analyzer Carlo Erba EA1108 analyzer. The IR spectra of the Schiff bases and their few metal complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000–250 cm^{-1} region in a KBr disc. The electronic spectra of the complexes were recorded in HPLC-grade DMSO solvent on a VARIAN CARY 50-BIO UV spectrophotometer in the region 200–1100 nm. The electrochemistry of all the complexes was recorded on a CHI1110A-electrochemical analyzer (made in the USA) in DMSO containing 0.05M $n\text{-Bu}_4\text{NClO}_4$ as the supporting electrolyte. Molar conductivity measurements were recorded on an ELICO-CM-82 T Conductivity Bridge with a cell having a cell constant of 0.51 and the magnetic moment was carried out by using Faraday balance.

Synthesis of the ligand

Synthesis of 7-Hydroxy-4-Methylcoumarin[1].

A mixture of resorcinol (0.2 mol) and ethylacetoacetate (0.2 mol) is cooled to 0-5 °C and conc. sulphuric acid (25 mL) is added gradually with constant shaking [17]. The reaction mixture is then kept in a refrigerator for 24 h. and poured into crushed ice with stirring. The separated solid is filtered, washed with water and recrystallized from ethanol as cream colored needles. Yield: 82%; MP: 185 °C.

Synthesis of 8-Formyl-7-Hydroxy-4-Methylcoumarin[2].

8-formyl-7-hydroxy-4-methylcoumarin is synthesized as described in the literature [17]. 7-hydroxy-4-methylcoumarin (0.03 mol) and hexamethylenetetramine (HMTA) (0.07 mol) in 50 mL glacial acetic acid was heated for 4-5 h. on water bath. To this, 75 mL of 20% HCl was added and the heating was continued for another 20 minutes. Then, the solution is cooled to room temperature and extracted with ether. The ether layer was evaporated and the remaining liquid portion was poured into crushed ice which resulted in the pale yellow colored solid. The product formed is filtered, dried and was recrystallized from hot ethanol. Yield: 16.36%; MP: 176-177°C.

Synthesis of Schiff base ligand[3].

The Schiff base has been synthesized by refluxing the reaction mixture of hot ethanolic solution (30 mL) of 2,4-dinitrophenyl hydrazine (0.01 mol) and hot ethanolic solution (30 mL) of 8-formyl-7-hydroxy-4-methylcoumarin (0.01 mol) for 4-5 h. with the addition of 2-3 drops of conc. hydrochloric acid [18]. Resulting mixture of Schiff base was filtered, washed with cold EtOH and recrystallized from EtOH.

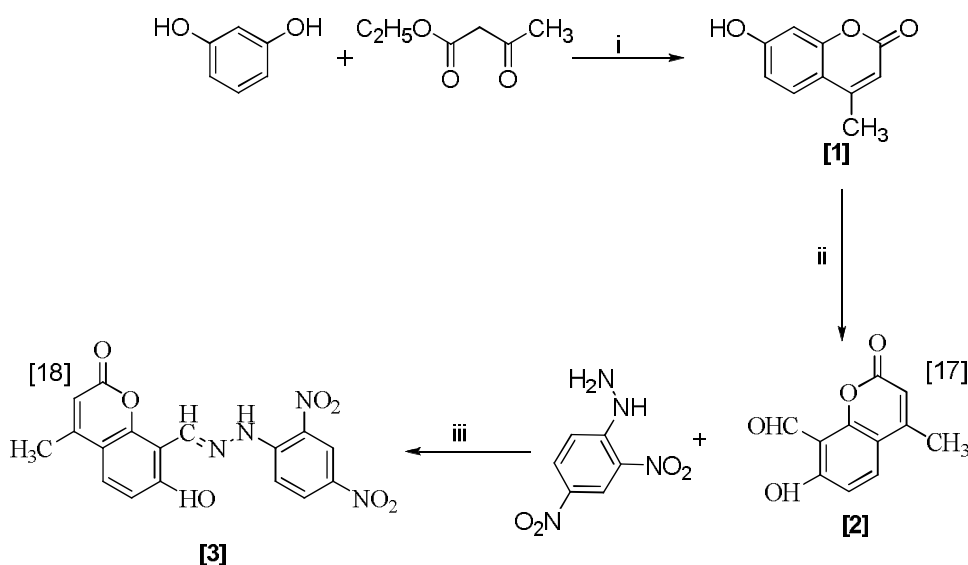
For $C_{37}H_{35}N_8O_{16}$

Anal. Calc. %. C. 53.03, H. 3.10, N. 14.21, O.29.01.

Found %. C.53.09, H. 3.12, N.14.25. O. 29.07.

IR data (KBr; ν , cm^{-1}): 2910-2920 br, 2310-2320 s, 1720-1725 s, 1475-1485 s, 1150-1155 v s,

Scheme;



i=Conc. H_2SO_4 , 0-5°C. ii= CH_3COOH , 4-5 h, HMTA(hexamethylenetetramine). iii=EtOH, 3-4 h.

Synthesis of metal complexes. An alcoholic solution of Schiff base (2 mmol) was refluxed with (1 mmol) of metal chlorides in ethanol on water bath for 2-3 h [19]. Then, to the reaction mixture (2 mmol) of sodium acetate was added and reflux was continued for 3h. The separated complex was filtered, washed thoroughly with water, ethanol and ether. Finally the complexes were dried in vacuum over fused $CaCl_2$.

Synthesis of $[\text{Mn}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$ (1a). Yield: 61%. M.P: 182-185 °C, Anal. Calcd for: $[\text{Mn}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$, Mn, 6.98; C, 49.02; H, 3.84; N, 12.21; O, 28.12. The new bands in the region of $\sim 685\text{-}695\text{ cm}^{-1}$ and $\sim 445\text{-}455\text{ cm}^{-1}$ in the metal complexes are assigned to stretching frequencies of $\nu(\text{M-N})$ and $\nu(\text{M-O})$ bonds respectively.

Synthesis of $[\text{Co}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$ (1b). Yield: 51%. M.P: 196-198 °C, Anal. Calcd for: $[\text{Co}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$, Co, 6.81; C, 48.31; H, 3.21; N, 12.07; O, 28.06. In the low-frequency region, two bands were observed for complexes at $\sim 671\text{-}674$ and $\sim 452\text{-}456\text{ cm}^{-1}$, which were attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively.

Synthesis of $[\text{Ni}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$ (1c). Yield: 53%. M.P: 205-208 °C, Anal. Calcd for: $[\text{Ni}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$, Ni, 6.83; C, 48.61; H, 3.45; N, 11.86; O, 28.14. In the low-frequency region, two bands were observed for complexes at $\sim 670\text{-}673$ and $\sim 448\text{-}452\text{ cm}^{-1}$, which were attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively.

Synthesis of $[\text{Cu}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$ (1d). Yield: 65%. M.P: 198-200 °C, Anal. Calcd for: $[\text{Cu}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$, Cu, 7.01; C, 48.12; H, 3.58; N, 12.10; O, 27.89. In the low-frequency region, two bands were observed for complexes at $\sim 664\text{-}670\text{ cm}^{-1}$ and $\sim 460\text{-}465\text{ cm}^{-1}$, which were attributed to $\nu(\text{M-N})$ and $\nu(\text{M-O})$, respectively.

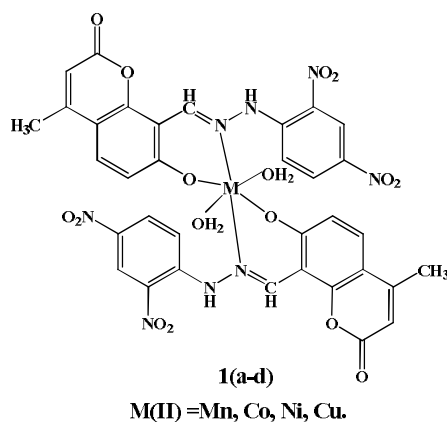


Figure-1; Proposed structure of metal complexes

BIOLOGICAL TESTING

Enzymatic Activity (Acetylcholinesterase activity (AChE))

Acetylcholine esterase activity was assessed as described by and modified by Briefly, claw muscles were homogenised on ice in 20 mM phosphate buffer, pH 7.0, 0.1% Triton X-100 (1/3 volume/weight) using an Ultra-Turax homogenizer and centrifuged for 20 min at 10000g. The acetylcholine esterase activity of 100 μL supernatant in presence of 0.01 M dithionitrobenzoic acid (DTNB) as reagent and 2.8 mM acetylthiocholine iodide (AcSCh) as substrate was then measured at 412 nm using a microplate reader at a controlled temperature of 20 °C. No attempt was made to distinguish between AChE and pseudo-cholinesterase. The activity of the enzyme was expressed in nmol of substrate hydrolysed per minute per milligram of protein.

Antioxidant activity

DPPH assay-The evaluation of antioxidant activity of newly synthesized compounds was done by DPPH radical scavenging activity assay. Internal standard BHA and the synthesized compounds of different concentrations were prepared in distilled ethanol, 1 mL of each

compound solutions having different concentrations (10 μ M, 25 μ M, 50 μ M, 100 μ M, 200 μ M and 500 μ M) were taken in different test tubes, 4 mL of 0.1 mM ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark room at RT for 20 min. A DPPH blank was prepared without compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using a UV-visible spectrophotometer and the remaining DPPH was calculated. The percent decrease in the absorbance was recorded for each concentration, and percent quenching of DPPH was calculated on the basis of the observed decreased in absorbance of the radical. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the formula:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_1) / A_0 \times 100]$$

Where A_0 is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound.

Antimicrobial activity

The in vitro biological screening effects of the investigated compounds were tested against the bacteria: *Escherichia coli*, *Bacillus subtilis* and *Ralstonia solanacearum* by the well-diffusion method, 24 using agar nutrient as the medium. The antifungal activities of the compounds were evaluated by the well-diffusion method against the fungi viz., *Aspergillus niger*, *Aspergillus flavus* and *Rhizoctonia bataicola* cultured on potato dextrose agar as medium. The stock solution (10^{-2} M) was prepared by dissolving the compounds in DMSO and the solutions were serially diluted to find MIC values. In a typical procedure, 25 a well was made on the agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated 24 h for bacteria and 72 h for fungi at 35°C. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration was noted.

RESULTS AND DISCUSSION

Chemistry. The synthetic strategies adopted for the synthesis of the intermediate and target compounds are depicted in the Scheme-1. A mixture of 8-formyl-7-hydroxy-4-methylcoumarin and 2,4-dinitrophenylhydrazine in 1:1 ratio in alcoholic medium containing 5-6 drops of concentrated HCl was refluxed for 3-4 h to form Schiff base [18]. The purity of the ligands was confirmed by elemental analyses, electronic spectra and IR spectral studies.

Physico-chemical techniques

Electrical Conductivity.

$$\Lambda_M = \frac{1000 \times \text{Cell Constant} \times \text{Specific Conductance}}{\text{Molar Concentration}}$$

Conductivity measurements of the complexes in HPLC grade DMSO solutions were made to verify the ionic formulation of the complexes. The molar conductivity of a solution was obtained from a following relation. The measurements were made by using a systronics conductivity meter 304 conductivity bridge provided with a dip-type conductivity cell having platinised platinum electrodes. The cell constant was determined by measuring the resistance of aqueous

KCl solutions, the specific conductivities of which were known accurately from literature [19]. The conductivity measurements of the complexes were carried out at 10^{-3} M in HPLC grade DMSO solvents.

The magnetic susceptibility measurements of the complexes were obtained at room temperature using Gouy balance. Pure $\text{Hg}[\text{Co}(\text{SCN})_4]$ was synthesized and used as calibration standard [20]. The effective magnetic moment values of the complexes are in table-1. The complexes are paramagnetic in nature. The magnetic moment values of complexes are in the range 1.29-5.85 BM, which suggests that the complexes have high spin octahedral geometry [21, 22]. The Mn(II), Co(II), Ni(II) and Cu(II) complexes are colored, stable and non-hygroscopic in nature. The complexes are insoluble in common organic solvents like chloroform, ethanol and methanol but soluble in DMF and DMSO. The elemental analyses shows that, the above complexes have 1:2 stoichiometry of the type $\text{ML}_2 \cdot 2(\text{H}_2\text{O})$ where L stands for a singly deprotonated ligands. The molar conductance values are too low to account for any dissociation of the complexes in DMSO, indicating the non-electrolytic nature of the complexes in DMSO shown in table-1.

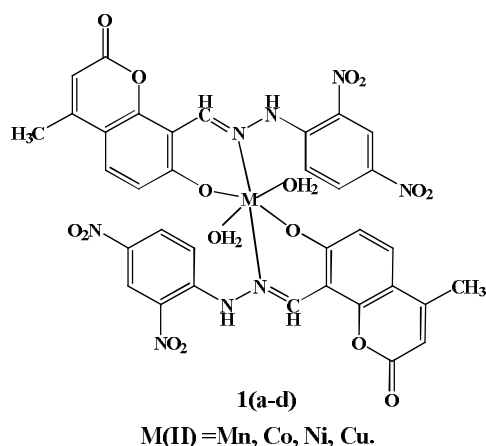


Figure-1. Proposed structure for metal complex

Table-1; Molecular weight, melting point, Elemental analysis, yield, magnetic measurements

Compounds	Mol. Formula	Mol. wt	M.p. °C	Calc. (found) (%)					Yield (%)	μ_{eff} (B.M.)	Mol. cond. ($\Omega\text{cm}^2\text{mol}^{-1}$)
				M	C	H	N	O			
L	$\text{C}_{17}\text{H}_{12}\text{N}_4\text{O}_7$	389.25	195-198	--	53.03(53.09)	3.10(3.12)	14.21(14.25)	29.01(29.07)	40	--	--
1a	$[\text{Mn}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	901.86	182-185	6.98(7.03)	49.02(49.11)	3.84(3.92)	12.21(12.30)	28.12(28.18)	61	5.81	28
1b	$[\text{Co}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	901.56	196-198	6.81(7.04)	48.31(48.40)	3.21(3.45)	12.07(12.12)	28.06(28.11)	51	3.75	23
1c	$[\text{Ni}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	898.41	205-208	6.83(7.01)	48.61(48.65)	3.45(3.51)	11.86(11.91)	28.14(28.20)	53	2.45	32
1d	$[\text{Cu}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	905.26	198-200	7.01(7.09)	48.12(48.15)	3.58(3.61)	12.10(12.14)	27.89(27.94)	65	1.29	36

Table-2; The Important Infrared Frequencies (in cm^{-1}) of Metal Complexes.

Compound	Coordinated $\nu(\text{H}_2\text{O})(\text{cm}^{-1})$	Lactonyl $\nu(\text{C}=\text{O})(\text{cm}^{-1})$	$\nu(\text{C}=\text{N})(\text{cm}^{-1})$	$\nu(\text{NH})(\text{cm}^{-1})$	Phenolic $\nu(\text{C}-\text{O})(\text{cm}^{-1})$	$\nu(\text{M}-\text{N})(\text{cm}^{-1})$	$\nu(\text{M}-\text{O})(\text{cm}^{-1})$
Ligand($\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16}$)	--	1720-1725	1475-1485	2910-2920	1150-1155	--	--
$[\text{Mn}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	3420-3430	1732-1740	1516-1525	2926-2930	1080-1085	685-695	445-450
$[\text{Co}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	3446-3450	1697-1700	1614-1624	2924-2931	875-880	671-674	452-456
$[\text{Ni}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	3450-3455	1705-1710	1620-1625	2930-2935	995-1005	670-673	448-452
$[\text{Cu}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	3425-3435	1702-1015	1595-1605	2932-2940	1050-1060	664-670	460-465

Table-3; Enzymatic Inhibition (%) of the Schiff base ligand and its metal complexes

Compounds No.	Complexes	% AchE. Inhibition
L	Ligand($\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16}$)	64 ± 0.25
1a	$[\text{Mn}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	72 ± 0.09
1b	$[\text{Co}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	81 ± 0.13
1c	$[\text{Ni}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	53 ± 0.14
1d	$[\text{Cu}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	91 ± 0.56

Table-4; Antioxidant activity (IC_{50}) of the Schiff base ligand and its metal complexes

Compound No.	Compounds	IC_{50} (μM)
L	Ligand ($\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16}$)	>310
1a	$[\text{Mn}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	209 ± 0.13
1b	$[\text{Co}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	121 ± 0.09
1c	$[\text{Ni}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	224 ± 0.53
1d	$[\text{Cu}(\text{C}_{37}\text{H}_{35}\text{N}_8\text{O}_{16})(\text{H}_2\text{O})_2]$	122 ± 0.21
Standard	Ascorbic acid	63 ± 0.05

Table-5; Antibacterial activity of the Schiff base ligand and its metal complexes (minimum inhibitory concentration $\times 10^{-2}$ M).

Compounds	E. coli	B. subtilis	R. solanacearum
Ligand(C ₃₇ H ₃₅ N ₈ O ₁₆)	5.2	11.0	06.0
[Mn(C ₃₇ H ₃₅ N ₈ O ₁₆)(H ₂ O) ₂]	4.5	10.3	04.6
[Co(C ₃₇ H ₃₅ N ₈ O ₁₆) (H ₂ O) ₂]	4.1	08.0	05.0
[Ni(C ₃₇ H ₃₅ N ₈ O ₁₆) (H ₂ O) ₂]	4.6	10.0	07.3
[Cu(C ₃₇ H ₃₅ N ₈ O ₁₆) (H ₂ O) ₂]	3.8	10.2	06.2
chloramphenicol	6.0	07.0	04.0

Table-6; Antifungal activity of the Schiff base ligand and its metal complexes (minimum inhibitory concentration $\times 10^{-2}$ M).

Compounds	A. niger	A. flavus	R. bataicola
Ligand(C ₃₇ H ₃₅ N ₈ O ₁₆)	6.0	5.4	5.1
[Mn(C ₃₇ H ₃₅ N ₈ O ₁₆)(H ₂ O) ₂]	3.1	3.5	4.5
[Co(C ₃₇ H ₃₅ N ₈ O ₁₆) (H ₂ O) ₂]	4.9	4.2	5.1
[Ni(C ₃₇ H ₃₅ N ₈ O ₁₆) (H ₂ O) ₂]	5.6	5.1	6.1
[Cu(C ₃₇ H ₃₅ N ₈ O ₁₆) (H ₂ O) ₂]	5.1	4.1	3.4
Nystatin	8.0	7.1	5.4

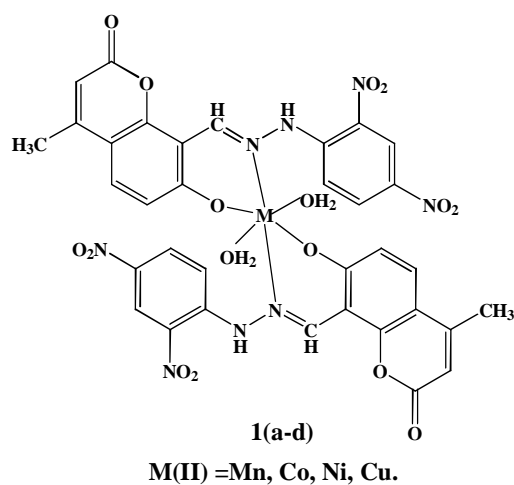


Figure-1; Proposed structure of metal complexes

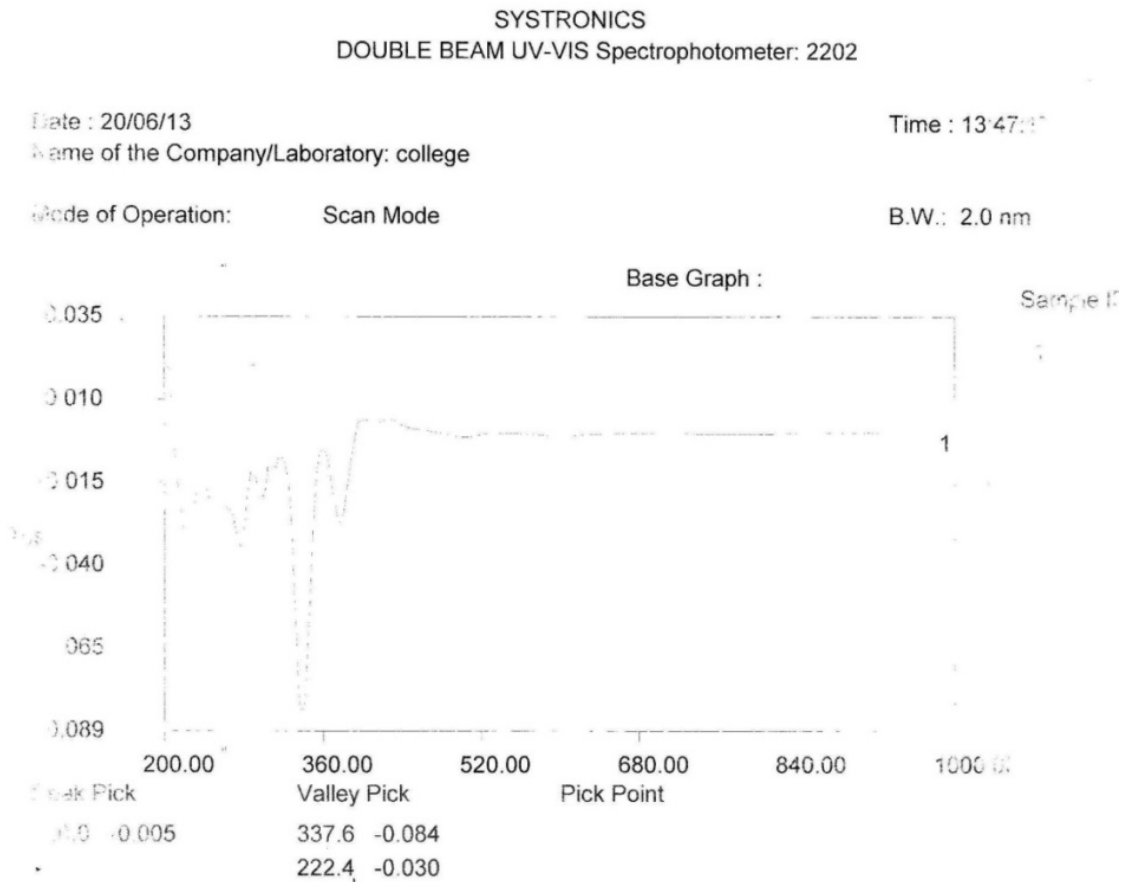


Figure-2; Electronic Absorption Spectrum of Schiff base

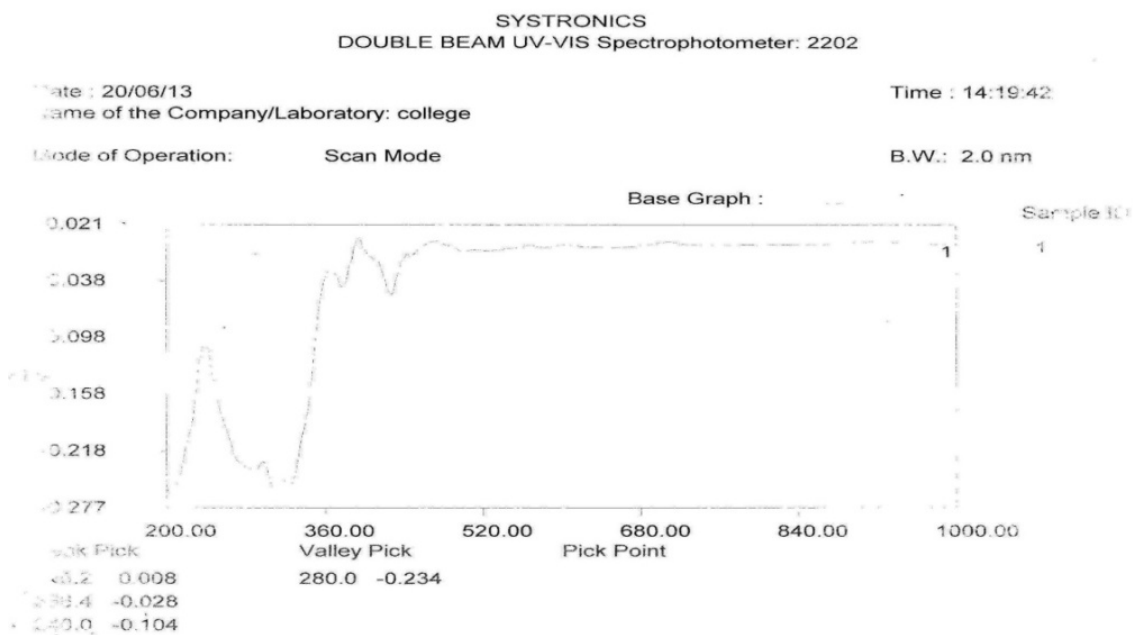


Figure-3; Electronic Absorption Spectrum of Co(II) Complex

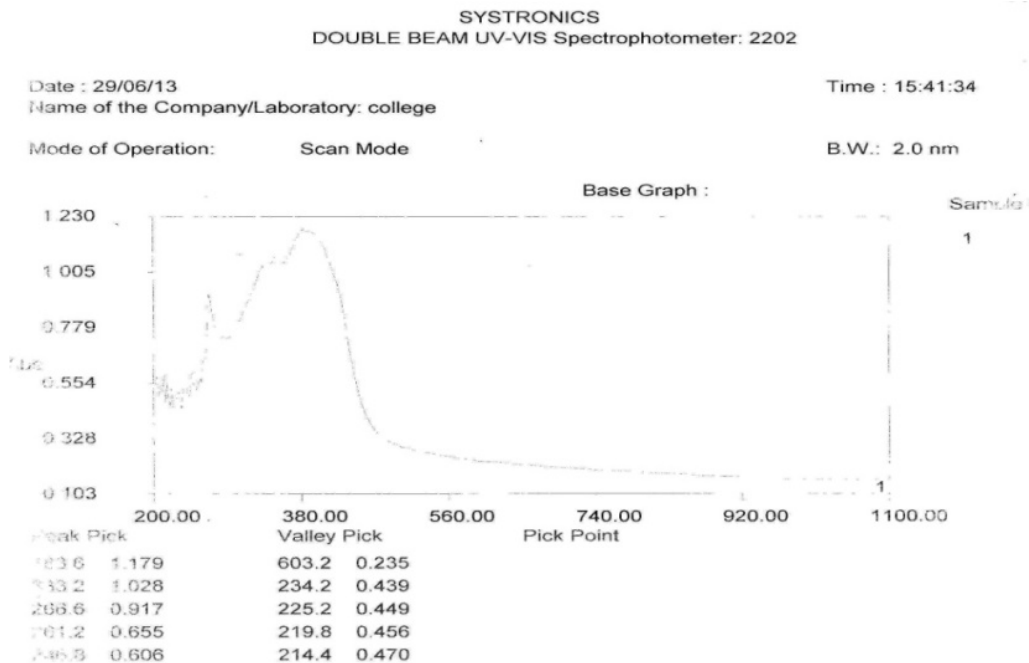


Figure-4; Electronic Absorption Spectrum of Ni(II) Complex

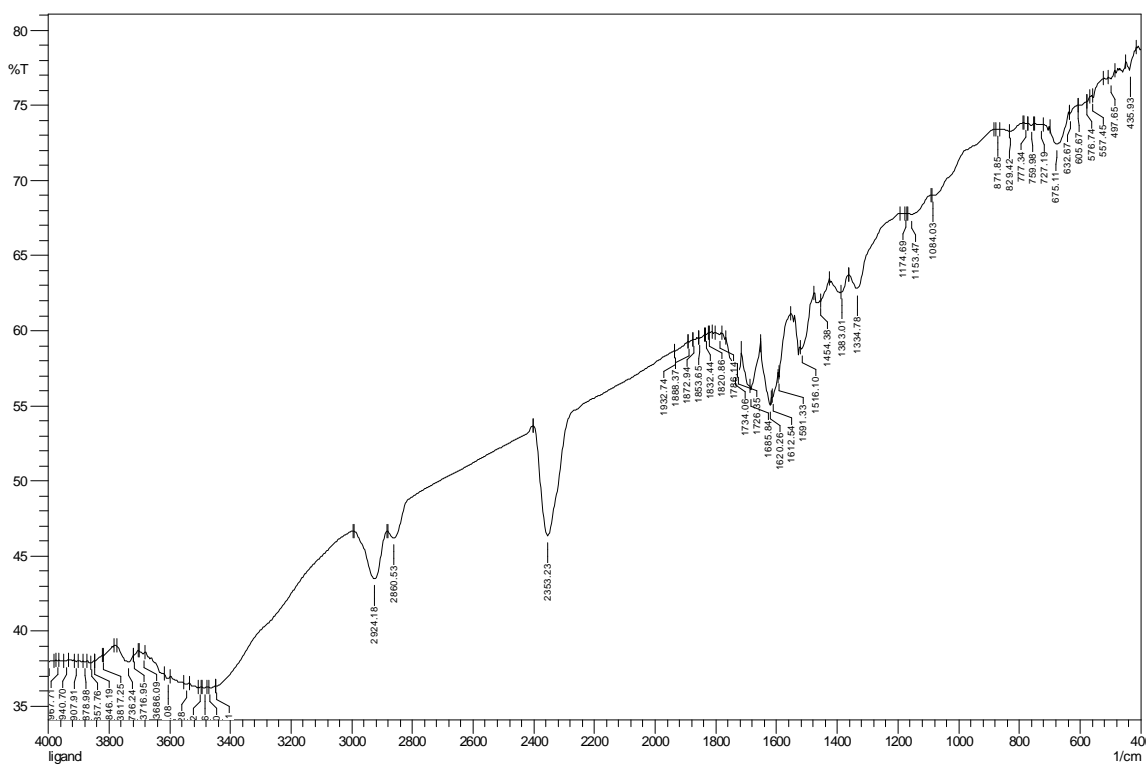


Figure-5; IR Spectrum of Schiff Base

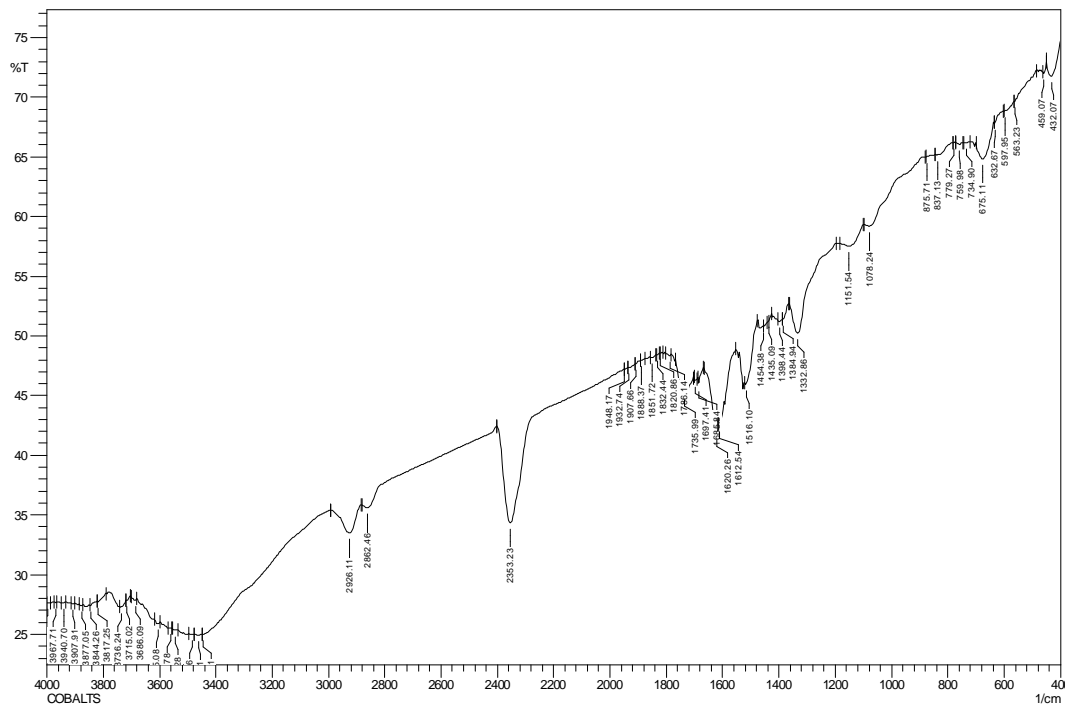


Figure-6; IR Spectrum of Co (II) Complex

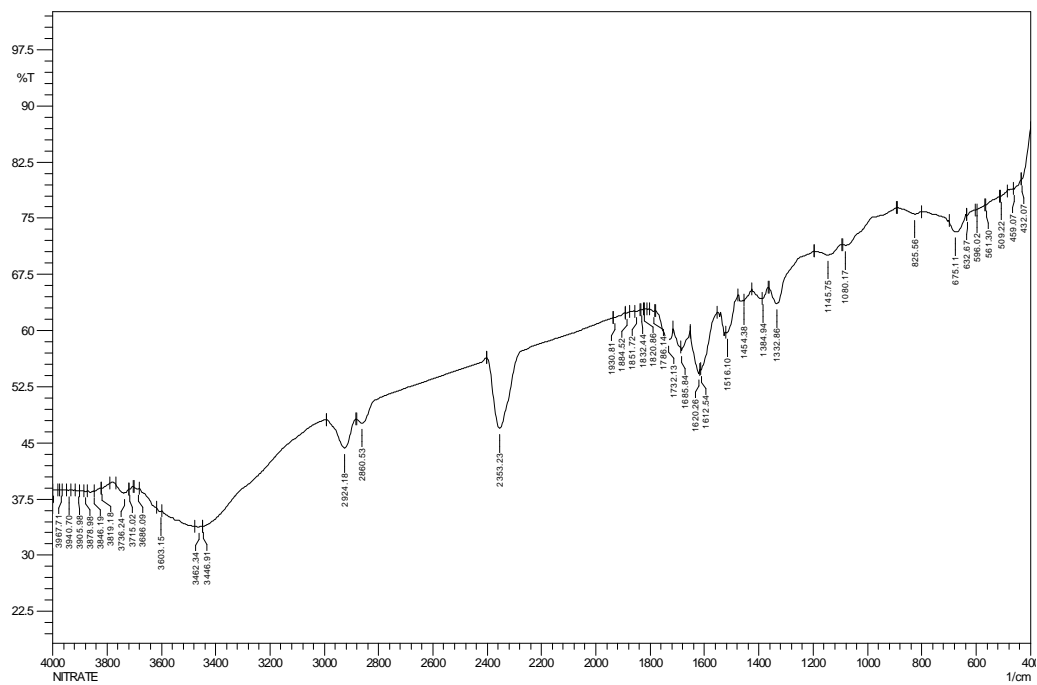


Figure-7; IR Spectrum of Ni (II) Complex

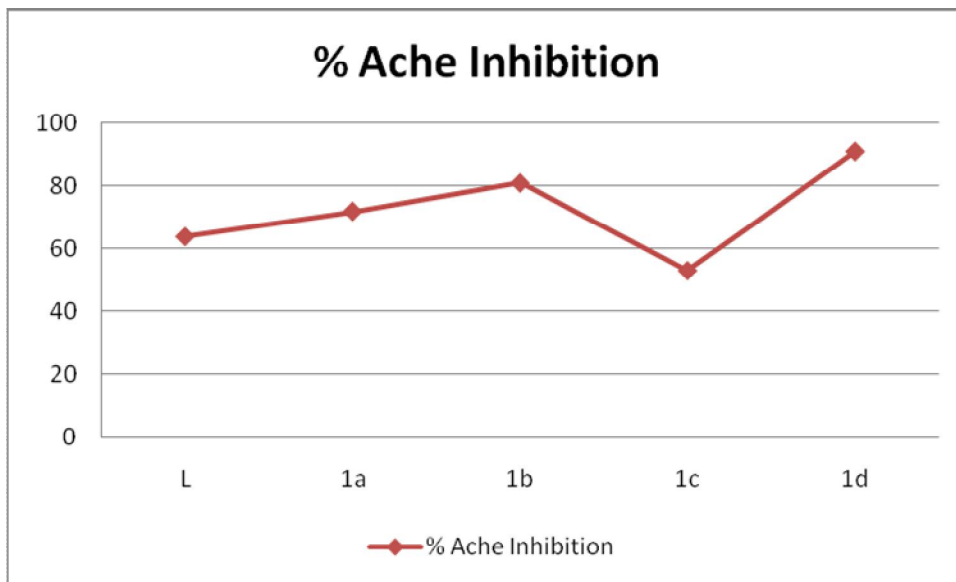


Figure-8; *Enzymatic Inhibition (%)* activity of the Schiff base ligand and its metal complexes

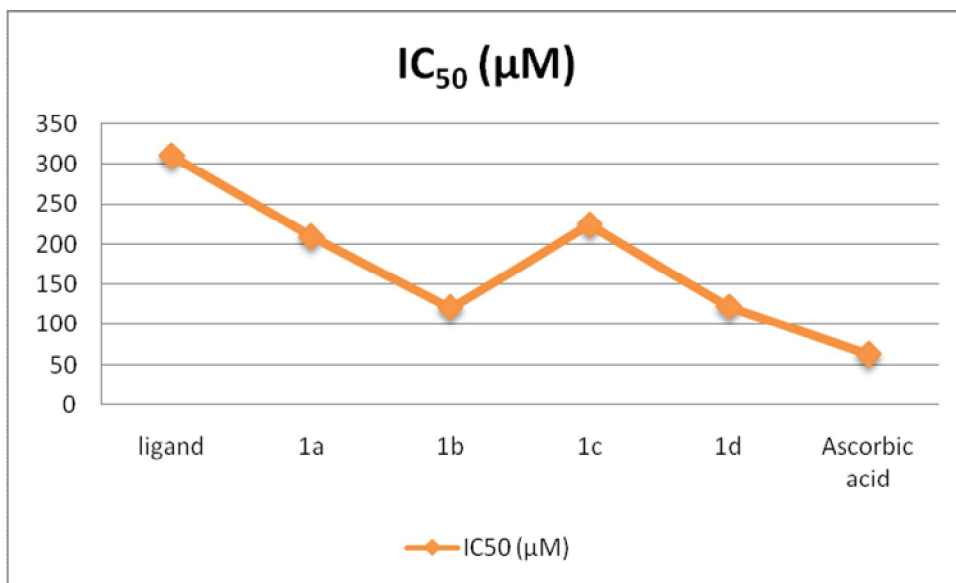


Figure-9; *Antioxidant activity* of the Schiff base ligand and its metal complexes

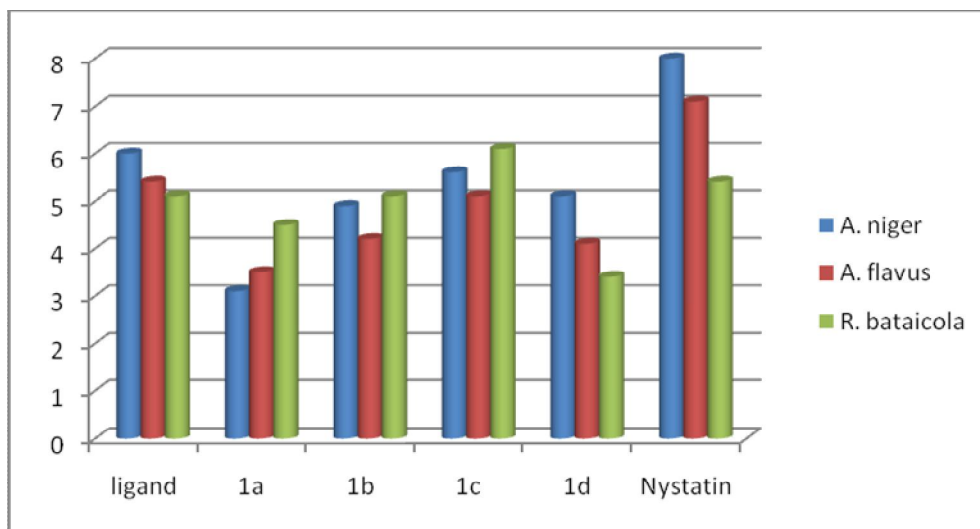


Figure-10; Antibacterial activity of the Schiff base ligand and its metal complexes

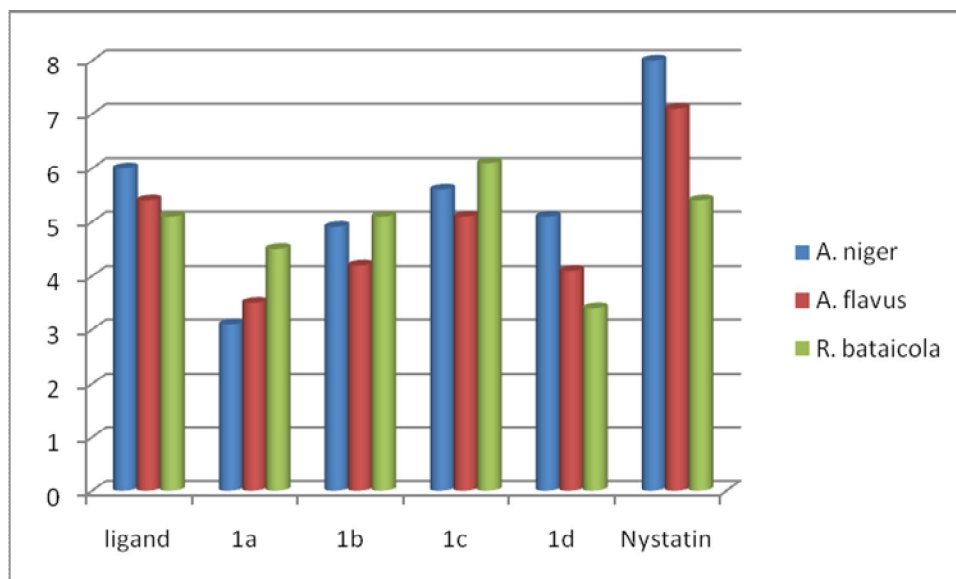


Figure-11; Antifungal activity of the Schiff base ligand and its metal complexes

Electronic Absorption Spectral Studies. The UV-Visible spectra in the region 200-1100 nm were measured by systronics double beam spectrophotometer 2202 model using calibrated quartz cells. Weighed samples were dissolved in appropriate solvents of HPLC grade at a concentration of 10^{-6} and 10^{-7} M. All the measurements were carried out at room temperature. The electronic absorption spectrum of the Schiff base is depicted in figure-2 exhibited two absorption bands around 340 nm and 420 nm respectively. The first band around 340 nm corresponds to $\Pi \rightarrow \Pi^*$ of the azomethine group and second band at 420 nm corresponds to $n \rightarrow \Pi^*$ transitions. The typical electronic absorption spectrum of the Co(II) complex assented in figures-3. The electronic spectra of Co(II) complexes exhibit absorption bands in the region $8000-10000 \text{ cm}^{-1}$ and $18000-20000 \text{ cm}^{-1}$ corresponding to the transitions ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F) (\nu_1)$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P) (\nu_3)$ respectively [23]. In the present investigation, brownish Co(II)

complexes shows the absorption bands around 240 nm and 384 nm corresponding to ν_1 and ν_3 transitions respectively. The ν_2 band is not observed due to strong proximity to ν_3 transition. These bands are characteristic of high spin octahedral Co(II) complexes. The spectrum is presented in the figure-3. The Ni(II) complex exhibited three bands at 246, 261 and 266 nm attributed to the ${}^3A_{2g} \rightarrow {}^3T_{2g}$ (ν_1), ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F) (ν_2) and ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (P) (ν_3) transitions respectively, which indicate octahedral geometry around Ni(II) ion and the spectra shown in figure-4. And also for Cu(II) and Mn(II) complexes results comparable with other reported complexes [24-26]. The electronic spectra of Cu(II) complexes display two prominent bands: a low-intensity broad band around $16,243\text{ cm}^{-1}$ is assignable to ${}^2E_g \rightarrow {}^2T_{2g}$ transition and another high-intensity band at $26,366\text{ cm}^{-1}$ is due to ligand \rightarrow metal charge transfer. On the basis of electronic spectra, the distorted octahedral geometry around the Cu(II) ion is suggested [27]. The Cu(II) complexes showed magnetic moment of 1.80–1.86 BM, which is slightly higher than the spin-only value (1.73 BM) expected for one unpaired electron, which offers the possibility of an octahedral geometry [28].

Infrared Spectral Studies. The Spectra of ligand and its metal complexes were obtained on a Shimadzu 8400-S, Japan, in the region $4000\text{--}350\text{ cm}^{-1}$. The typical infrared spectrum of the Schiff base has been reproduced in figure-5. The most important IR bands of Schiff base exhibited a broad band at 3489 cm^{-1} , strong band at 1683 & 1622 cm^{-1} assigned to H-bonded -OH stretching, $\nu(\text{C=O})$ lactonic carbonyl and azomethine $\nu(\text{C=N})$ vibrations respectively [28]. A medium band around 975 cm^{-1} is characterized for ν (O-C-O) vibrations. A broad band in the range 2924 cm^{-1} is ascribed to the stretching vibration of $\nu(\text{NH})$ [28]. The medium intensity bands at 1050 and 1150 cm^{-1} are attributed to $\nu(\text{N-N})$ and $\nu(\text{C-O})$ vibrations, respectively [29]. The IR spectra of the metal complexes clearly indicate the bonding mode of Schiff base with the metal ions. The prominent infrared spectral data are presented in the table-2 and the some of IR spectra of the representative Co(II), Ni(II) complexes are shown in figures-6 and figure-7 respectively.

In comparison with the spectra of the Schiff base, the metal complexes exhibited the band of $\nu(\text{C=N})$ in the region 1612 cm^{-1} ; showing the shift of band to lower wave numbers indicating that, the azomethine nitrogen is coordinated to the metal ion. The disappearance of the broad band due to H-bonded -OH stretching in the spectra of the metal complexes gives evidence for the formation (M-O) bonds via deprotonation. Further, the high intensity band due to phenolic $\nu(\text{C-O})$ appeared as 1080 cm^{-1} . The new bands in the region of 451 and 694 cm^{-1} in the metal complexes are assigned to stretching frequencies of (M-O) and (M-N) bonds respectively. The unaltered position due to lactonyl $\nu(\text{C=O})$ in all the metal complexes confirms its non involvement in coordination. Thus, the IR spectral data provide strong evidence for the complexation of the potentially bidentate Schiff base [30].

Enzymatic Activity (Acetylcholine esterase activity (AChE)). In present investigation, we studied the Ache enzyme inhibition activity percentage of our newly prepared ligand and metal complexes for the discovery of new acetylcholinesterase inhibitors. Various structural studies reveals that the specific positioning of the nitrogen atom and octahedral geometry of the metal complexes in relation to the aromatic ring will shows better inhibition activity [34] and it is essential for the strong affinity between the antagonists and the receptor. Examined the overlap factor (HOMO) through an indirect approach receptor mapping [31]. According to his calculations, the metal complexes of 1a, 1b and 1d are very active compounds and the metal

complexes of 1c and ligand shows moderate enzymatic inhibition activity the data shown in table- 3 and figure-8.

Antioxidant activity.

DPPH Radical Scavenging Activity. The antioxidant activity of ligand as well as metal complexes is presented in table-4 and figure-9. At the different concentration of 10, 25, 50, 100, 200 and 500 μM , few complexes showed a much stronger DPPH scavenging (>80%) than free Ascorbic acid (63%). The vitamin C showed 63% of DPPH scavenging property. The marked antioxidant activity of ligand [L] and metal complexes 1a and 1c in comparison to free Ascorbic acid, could be due to the coordination of metal after complexation of the system, increasing its capacity to stabilize unpaired electrons and Thereby, to scavenge free radicals. And remain complexes of 1b and 1d shows moderate activity of scavenging property [32].

Antimicrobial activity.

The minimum inhibitory concentration (MIC) values of the compounds against the respective strains are summarized in table-5, table-6, figure-10 and figure-11. The antimicrobial screening results of all the synthesized ligand and their metal complexes exhibited antimicrobial properties. It is important to note that the metal complexes exhibited a more inhibitory effect compared to their respective parent ligands. The enhanced activity of the complexes over the ligand can be explained on the basis of chelation theory [33]. It is known that chelation makes the ligand a more powerful and potent bactericidal agent, thus killing more of the bacteria than the ligand. The enhancement in the activity may be rationalized on the basis that ligands mainly possess an azomethine (C = N) bond. It has been suggested that ligands with hetero donor atoms (nitrogen and oxygen) inhibit enzyme activity, since the enzymes that require these groups for their activity appear to be especially more susceptible to deactivation by metal ions on coordination. It is observed that, in a complex, the positive charge of the metal ion is partially shared with the hetero donor atoms (nitrogen and oxygen) present in the ligand, and there may be π -electron delocalization over the whole chelating system [34]. Thus the increase in the lipophilic character of the metal chelates favors their permeation through the lipid layer of the bacterial membranes and blocking of the metal binding sites in the enzymes of microorganisms. Other factors, namely solubility, conductivity and bond length between the metal ion and the ligand, also increase the activity. The increase in the activity of metal complexes against fungi is due to the formation of a hydrogen bond between the azomethine nitrogen atom and active centers of the cell constituents, resulting in interference with the normal cell process.

CONCLUSION

Based on these studies, the newly synthesized ligands and their complexes were characterized by various spectral studies and analytical data. The coordinating ability of the ligands was proved in complexation reactions with Mn(II), Co(II), Ni(II) and Cu(II) ions. In all the complexes ligand act as a tridentate chelate around the metallic ion with 2 compartments and provide ONO donating sites to each metal ion in both compartments. On the basis of different techniques, it is proposed that, the metal complexes have octahedral geometries and the Schiff base act as a versatile tridentate ligand. The bonding of ligand to metal ion was confirmed by the analytical and spectral studies. All these observations put together lead us to propose the following

structure for the Mn(II), Co(II), Ni(II) and Cu(II) complexes. The biological activity results show that all the complexes exhibited effective activity when compared to their respective ligand.

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