

Synthesis, characterization, antimicrobial, anticancer and antidiabetic activity of new Manganese (II), Nickel (II) and Cobalt (II) complexes with Salicylaldehyde-4-chlorobenzoylhydrazone.

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ABSTRACT : The new Schiff base ligand Salicylaldehyde-4-Chlorobenzoylhydrazone (HL) and its Mn(II), Co(II) and Ni(II) metal complexes were synthesized and characterized by various spectroscopic (UV-vis, IR, NMR), thermal and magnetic susceptibility measurement. The ligand (HL) was synthesized by condensation of Salicylaldehyde and 4-Chlorobenzhydrazide. On the basis of electronic spectral data and magnetic susceptibility measurement the octahedral geometry has been proposed for all the complexes. The ligand and metal complexes have been screened for their antimicrobial activities against bacteria *Staphylococcus aureus*, *Escherichia coli* and antifungal activity against the fungi *Aspergillus niger* and *Candida albicans*. Further the material was also screened for anticancer activity on human cancer cell lines such as breast (MCF7) and colon (HT29) by sulforhodamine-B (SRB) calorimetric assay. Interestingly, all these complexes showed significant anticancer activity. Also, antidiabetic activity of complexes determined by DNSA method.

KEYWORDS: Schiff base, Salicylaldehyde, 4-Chlorobenzhydrazide, Antimicrobial activity, Anticancer activity and Antidiabetic activity.

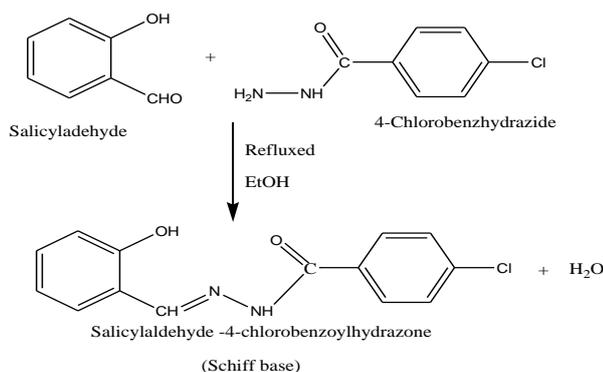
I. INTRODUCTION

Hydrazone compounds are found to be a very important class of compounds because they are involved in number of biological activities, such compounds are used as antiviral, anti-inflammatory agent [1], bacteriostats, fungicides, algacides, herbicides, antidepressant [2], anticonvulsants [3], chelating agent for neurodegenerative disorders, also inhibitor for DNA synthesis and cell growth in a variety of human and rodent cell lines [4]. Benzhydrazones and its derivatives are used as a catalyst in various chemical reactions [5]. They are also used in various optoelectronic devices [6]. In analytical chemistry hydrazones are used for selective metal extracting agent, spectroscopic determination of transition metal ion, gravimetric determination of metal ion, also spot test reagent for determination of metal ion [7]. It has been reported that hydrazones can be used as sterilant for houseflies and against cockroach. Many hydrazones have been used as an effective corrosion inhibitor of metal [8]. They are also used in luminescent probes [9] as well as molecular sensor [10].

Large number of Schiff base compounds and their metal complexes have been synthesized and structurally characterized. Various classes of Schiff bases that can be prepared by condensation of different type of hydrazide with carbonyl compounds are very popular due to diverse chelating ability. Several transition metal complexes have been screened for their medicinal properties [11-12]. The first row transition metals have attracted much attention due to their biological importance. These factors prompted us to carry out a study on synthesis of Schiff base and its complexes with Ni(II), Co(II), Mn(II) metal ions. In this paper we report the synthesis of new Schiff base Salicylaldehyde-4-Chlorobenzoylhydrazone (HL) and its ligation behavior with Mn(II), Ni(II) and Co(II) metals. The synthesized ligand and metal complexes were characterized by various spectroscopic (UV-vis, IR, NMR), thermal and magnetic susceptibility measurement. They are screened for their antimicrobial activity against bacteria *S. aureus*, *E. coli* and fungi *A. niger* and *C. albicans*. Further they are investigated for anticancer activity on human cancer cell lines such as breast (MCF7) and colon (HT29) by sulforhodamine-B (SRB) calorimetric assay. Further Materials are screened for their antidiabetic activity by DNSA method.

II. MATERIALS AND METHODS

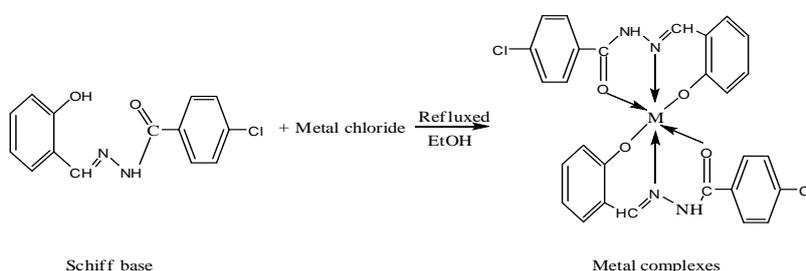
All reagents used were pure AR grade such as Salicylaldehyde, 4-Chlorobenzhydrazide, Cobalt chloride (CoCl₂·2H₂O), Nickel chloride (NiCl₂·6H₂O) and Manganese chloride (MnCl₂·6H₂O). The solvents used were Ethanol, DMSO etc. The synthesis of Schiff base (HL) is shown in Scheme 1



Scheme 1

Scheme 1: Synthesis of Schiff base

The Salicylaldehyde and 4-Chlorobenzhydrazide were dissolved in ethanol (30 ml) separately in 1:1 molar ratio. The ethanolic solutions were mixed together. The mixture was refluxed on water bath for an hour. On cooling, a crystalline compound was separated by filtration and the crystals were washed with ethanol and anhydrous diethyl ether and dried over anhydrous CaCl_2 [13]. A ligand Salicylaldehyde-4-Chlorobenzoylhydrazone was dissolved in (30 ml) ethanol and added to ethanolic solution (30 ml) of metal salt. The metal-ligand molar ratio was (1:1). The mixture was refluxed for 2 hrs. On cooling, a crystalline complex was separated by filtration and the crystals were washed and dried as above [14]. The synthesis of metal complexes is shown in Scheme 2.



Where $M = \text{Ni(II)}, \text{Co(II)}, \text{Mn(II)}$.

Scheme 2: Synthesis of metal complexes

Schiff base (HL) and their Mn(II) , Ni(II) and Co(II) complexes synthesized were stable at room temperature and soluble in common solvents like methanol, DMSO etc. The synthesized ligand and the metal complexes were characterized by elemental analysis and spectral analysis. Biological activity of the ligand and its metal complexes were also studied. Based on their elemental and spectral studies the geometry of the synthesized compounds has been elucidated.

III. RESULTS AND DISCUSSION

By the elemental analysis, the stoichiometry of ligand and their metal complexes is confirmed. The elemental analysis of ligand and the metal complexes are found in agreement with the proposed structure of ligand and the metal complexes. Analytical and Physicochemical data are listed in Table 1

Compounds (Empirical formula)	Molecular Weight	Colour	M.P.($^{\circ}\text{C}$)	%Observed (Theoretical)			
				C	H	N	Metal
HL $\text{C}_{14}\text{H}_{11}\text{N}_2\text{O}_2\text{Cl}$	274.05	Light yellow	158	61.10 (61.15)	4.15 (4.1)	10.10 (10.15)	--
$[\text{Mn}(\text{HL})_2]$ $\text{C}_{28}\text{H}_{20}\text{MnN}_4\text{O}_4\text{Cl}_2$	601.03	Yellow	>250	55.93 (55.90)	3.5 (3.3)	9.38 (9.31)	9.15 (9.13)
$[\text{Co}(\text{HL})_2]$ $\text{C}_{28}\text{H}_{20}\text{CoN}_4\text{O}_4\text{Cl}_2$	605.01	Brown	>250	55.60 (55.58)	3.20 (3.3)	9.1 (9.2)	9.74 (9.74)
$[\text{Ni}(\text{HL})_2]$ $\text{C}_{28}\text{H}_{20}\text{NiN}_4\text{O}_4\text{Cl}_2$	604.79	Green	>250	55.40 (55.55)	3.27 (3.3)	9.21 (9.25)	9.69 (9.7)

Table 1. Analytical and Physicochemical data of Schiff base (HL) and its metal complexes.

The electronic spectra and magnetic moment are very useful in the evaluation of results obtained by other methods of structural investigation. Information regarding the geometry of the complexes of Co(II), Ni(II) and Mn(II) ions was obtained from electronic spectral studies and magnetic moments. The electronic spectra of ligand and their metal complexes were recorded at room temperature using DMSO as a solvent. Electronic spectrum of ligand shows strong absorption band in region 300nm but in complexes they are slightly shifted to higher frequency, these are $\pi \rightarrow \pi^*$ transition [15]. Band in the region 330 nm can be assigned to the $n \rightarrow \pi^*$ transition of the azomethine group of ligand, in the spectra of the complexes, the bond of azomethine are shifted to lower frequency indicating that the azomethine nitrogen atom is involved in coordination to the metal ion. Very low intensity absorption bands associated with d-d transition of Mn(II) complex, it exhibit band at 375nm, 420nm and 523nm assigned $[^6A_{1g} \rightarrow ^4E_g(G)]$, $[^6A_{1g} \rightarrow ^4T_{2g}(G)]$ and $[^6A_{1g} \rightarrow ^4T_{1g}(G)]$ transition respectively [16]. Co(II) complex exhibit band at 435nm $[^4T_{1g} \rightarrow ^4T_{1g}(P)]$, 466nm $[^4T_{1g} \rightarrow ^4A_{2g}(F)]$ and 738nm $[^4T_{1g} \rightarrow ^4T_{2g}(F)]$. Ni (II) complexes exhibits three band at 430nm, 690nm and 950nm are due to $[^3A_{2g} \rightarrow ^3T_{1g}(P)]$, $[^3A_{2g} \rightarrow ^3T_{1g}(F)]$, $[^3A_{2g} \rightarrow ^3T_{2g}(F)]$ transitions respectively, indicating an octahedral geometry of metal complexes [17].

The magnetic moment obtained at the room temperature indicates paramagnetism. The Mn(II) complex shows magnetic moment of 5.99 BM, the spin free octahedral complexes of Mn(II) are reported to exhibit magnetic moment in the range of 5.80-6.20 BM [18]. Hence, the observed magnetic moment for the Mn(II) complex under study indicates that, it has an octahedral configuration. The Ni(II) complex shows magnetic moment of 3.18 BM, the magnetic moment of octahedral Ni(II) complexes are reported to exhibit magnetic moment in the range of 3.24 BM [19], indicating spin orbital coupling contribution from $^3A_{2g}$ and higher $^3T_{2g}$ states. Hence, the observed magnetic moment for the Ni(II) complex suggests that it may have octahedral geometry. The magnetic moment value observed for Co(II) complex is 4.60 BM indicates that it has an octahedral configuration [20-21]. Thus the electronic spectral data and magnetic moment data support the octahedral geometry for Mn(II) and Ni(II) and Co(II) complexes.

Compounds	$\nu(\text{NH})$ cm^{-1}	Intra Molecular H Bonding cm^{-1}	$\nu(\text{C}=\text{N})$ cm^{-1}	$\nu(\text{C}=\text{O})$ cm^{-1}	$\nu(\text{C-O})$ cm^{-1}	$\nu(\text{M-O})$ cm^{-1} phenolic	$\nu(\text{M-O})$ cm^{-1} ketonic	$\nu(\text{M-N})$ cm^{-1}
HL	3216	3065	1624	1642	1200	---	---	---
[Mn(HL) ₂]	3150	---	1614	1622	1300	445	350	425
[Co(HL) ₂]	3170	---	1613	1625	1317	447	340	420
[Ni(HL) ₂]	3180	---	1605	1629	1327	440	340	440

Table 2. IR spectral data (cm^{-1}) of Schiff base (HL) and its metal complexes.

The IR spectra of complexes are compared with those of ligand in order to determine the coordination sites that may be involved in chelation. In case of ligand a sharp band at 1624cm^{-1} corresponds to an azomethine stretching frequency and the band at 1642cm^{-1} is due to amide carbonyl. This suggests the existence of ligand in keto form in the solid state. The -NH stretching vibrations appear at 3216cm^{-1} and phenolic -OH vibration appear at 3065cm^{-1} . The C-O stretching and O-H bending vibrations are found around 1200cm^{-1} , 1352cm^{-1} respectively [22-23]. The absence of -OH stretching bands in IR spectra of all complexes, clearly indicates the coordination of ligand in its deprotonated form [24]. The decrease in $\nu(\text{C}=\text{O})$ stretching band vibrations upon coordination with Mn(II), Co(II), Ni(II) complexes in the range $1629-1622\text{cm}^{-1}$ clearly indicates its coordination in keto form. Strong bands in the range $1614-1605\text{cm}^{-1}$ for complexes are assigned to $\nu(\text{C}=\text{N})$ stretching which is low as compared with the ligand revealing its coordination to the metal ion [25]. The $\nu(\text{C-O})$ stretching vibration of phenolic shifted to higher frequency and appears in the region $1300-1327\text{cm}^{-1}$ in the complexes. In far-infrared spectral region the Mn(II), Co(II) and Ni(II) complexes exhibit bands at $440-447\text{cm}^{-1}$, $340-350\text{cm}^{-1}$ and $420-440\text{cm}^{-1}$, which are assigned to $\nu(\text{M-O})$ phenolic, $\nu(\text{M-O})$ ketonic and $\nu(\text{M-N})$ vibration respectively [26]. Two or three bands appearing in the region $1548-1486\text{cm}^{-1}$ may be assigned to various stretching modes such as $\nu(\text{C}=\text{C})$ vibration. Therefore, from the IR spectra, it is concluded, that ligand has coordinated through carbonyl oxygen, azomethine nitrogen and phenolic oxygen via deprotonation. The typical IR of Schiff base (HL) is shown in Figure 1.

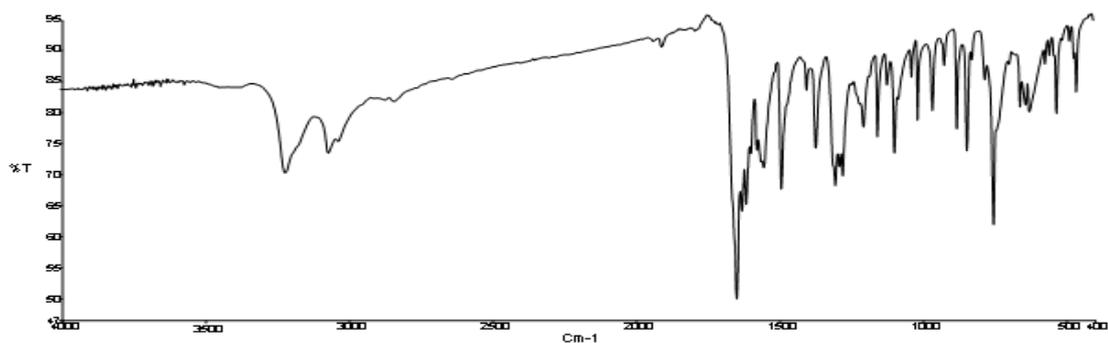


Figure 1. IR spectra of Schiff base (HL).

The $^1\text{H-NMR}$ spectra of the Schiff base (HL) shows D_2O exchangeable broad singlet at 11.995, 11.403 ppm integrating for one proton each, assigned to $-\text{OH}$ and $-\text{NH}$ respectively. The $^1\text{H-NMR}$ spectra of Schiff base (HL) the shows multiplet in the range 7.90-6.82 ppm due to the aromatic protons. The $-\text{N}=\text{CH}$ proton of Schiff base (HL) exhibit singlet in the region 8.58 ppm. $^1\text{H-NMR}$ of Schiff base (HL) is shown in Figure 2.

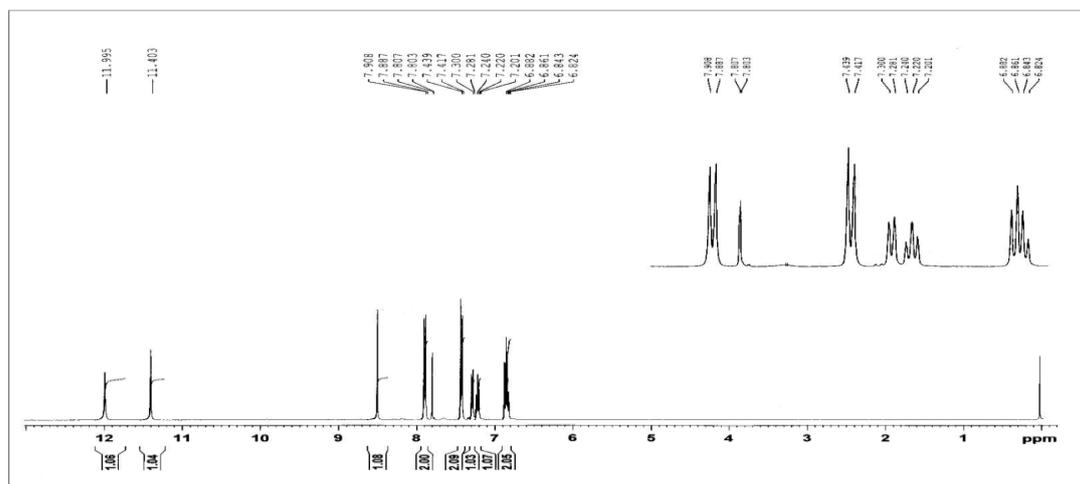


Figure 2. NMR spectra of Schiff Base (HL)

Thermal studies of complexes have been undertaken to know the presence or absence of coordinated water molecule and to confirm position well. The thermogram of Manganese complex is shown in Figure 3.

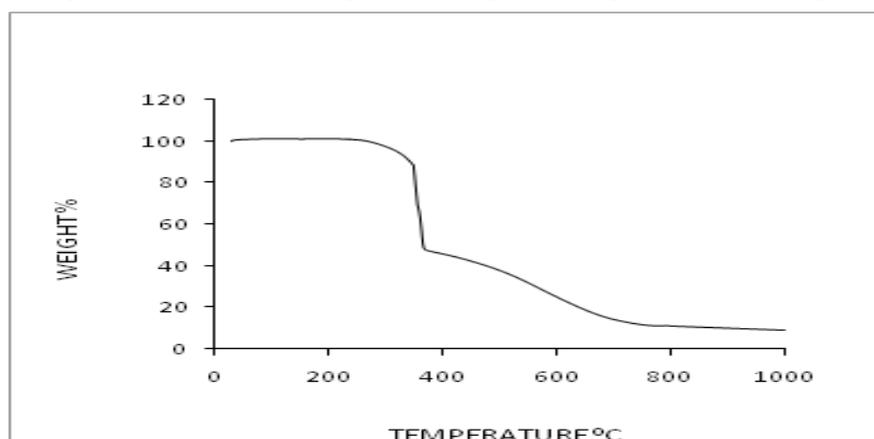


Figure 3. Thermogram of Mn(II) complex.

In case of Mn(II), Co(II) and Ni(II) complexes, no weight loss was observed upto 240 $^{\circ}\text{C}$ indicate the absence of lattice held or coordinated water molecules, weight loss of 90.80%, 90.20% and 90.25% respectively correspond

to the loss of two ligand molecules. The graph obtained after heating above 510⁰C corresponds to the formation of stable metal oxide [27-28]. Molar conductivities of metal complexes in DMSO at 10⁻³ M were very low (10.4-14.6 Ohm⁻¹cm²mol⁻¹) indicating the non electrolytic nature of complexes [29]. The antibacterial and antifungal activity of the Schiff base and their metal complexes was tested on bacteria such as *S. aureus*, *E. coli* (gram negative) and fungi *A. niger*, *C. albicans*. The method used for antibacterial activity was Agar Well-Diffusion method [30] and for antifungal activity was Agar-Ditch method [31]. The stock solution 500 µg/ml prepared and used to prepare concentrations of 20,40,60,80,100 µg/ml. The bacteria and fungi were inoculated on the surface of Nutrient agar and Sabouraud's agar respectively. The various concentrations compounds were inoculated in the wells and ditch prepared on the agar plates. The plates were incubated at 37⁰C for 24 hrs and 48 hrs for bacteria and fungi respectively. In order to clarify the effect of DMSO for its antimicrobial activity by agar plate assay, separate studies were carried out with DMSO and showed no activity against any bacteria and fungi. The results are as shown in the Table 3. Metal complexes in general exhibited better antibacterial and antifungal activity than ligand. Ni(II) complex exhibited highest antimicrobial activity among compound tested.

Compounds	Conc.µg / ml.	<i>S.aureus</i>	<i>E.Coli</i>	<i>C.albicans</i>	<i>A. niger</i>
HL	20	-	+	+	+
	40	---	+	+	+
	60	+	+	+	+
	80	+	+	+	++
	100	+	++	++	++
[Mn(HL) ₂]	20	+	+	+	+
	40	+	+	+	+
	60	+	+	+	+
	80	+	+	++	++
	100	++	++	++	+++
[Co(HL) ₂]	20	+	+	+	+
	40	+	+	+	+
	60	+	+	++	+
	80	++	++	++	++
	100	++	++	+++	++
[Ni(HL) ₂]	20	+	+	+	+
	40	+	+	+	+
	60	+	++	++	++
	80	++	+++	++	+++
	100	+++	+++	+++	+++
Inhibition:	- ve = Inactive, (Less than 0.5 cm)			+ ve = Weakly active (0.5-1 cm)	
	+ + ve = Moderately active, (1 to 2cm)			+ + + ve = Highly active (more than 2cm)	

Table 3 Antimicrobial activity data of Schiff base (HL) and its metal complexes.

The anticancer activity of the ligand (HL) and its Mn(II), Co(II) and Ni(II) complexes was determined by sulforhodamine -B colorimetric assay. The cell lines were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS) and 2 mM L-glutamine at 37 °C in a humidified atmosphere of 5% CO₂. About 5×10³ cells/well were seeded in 96-well micro titer plate using a culture medium. After 24hrs, Schiff base (HL) and its Mn(II), Co(II) and Ni(II) their metal complexes at the concentration of 10, 20, 40 and 80 µg mL⁻¹. Molar was added to respective wells and incubated for 48 hrs. After incubation the sulforhodamine-B assay was performed [32-33]. Ligand is resistant to both human cancer cell lines such as breast (MCF7) and colon (HT29) with GI50 of 52.2 µM and 94 µM respectively.

While Ni(II) complexes exhibit super *in vitro* anticancer activity against both breast (MCF7) and colon (HT29) cancer cell lines with IC₅₀ value of <0.1 µM, which is comparable to Adrinamycin with IC₅₀ of <0.1 µM in same assay. Mn(II) complex shows moderate activity for breast (MCF7) cell line with IC₅₀ value of 11.1 µM

and resistant to the colon (HT29) cell line with IC50 value of 84.9 μM also Co(II) complexes exhibit super in vitro anticancer activity against breast (MCF7) cell line with IC50 value of $<0.1 \mu\text{M}$ and weak activity for colon cell line with IC50 value of $35 \mu\text{M}$. Result of cytotoxicity of ligand (HL) and its complexes on human breast (MCF7) and Colon Cancer Cell Line (HT29) are shown in Table 4

Cell line used	$\mu\text{Molar drug concentrations}$ calculated from graph		
	LC50	TGI	GI50*
MCF7			
HL	>100	>100	52.2
[Mn(HL) ₂]	>100	>100	11.1
[Ni(HL) ₂]	>100	49.6	< 0.1
[Co(HL) ₂]	>100	>100	< 0.1
ADR	>100	45.7	< 0.1

Cell line used	$\mu\text{Molar drug concentrations}$ calculated from graph		
	LC50	TGI	GI50*
HT29			
HL	>100	>100	94
[Mn(HL) ₂]	>100	>100	84.9
[Ni(HL) ₂]	>100	<0.1	<0.1
[Co(HL) ₂]	>100	98.2	35.0
ADR	>100	22.2	<0.1

Table 4: Cytotoxicity of Schiff base (HL) and Mn(II), Ni(II) and Co(II) complexes on Human Breast Cancer Cell Line (MCF7)

Table 4: Cytotoxicity of Schiff base (HL) and Mn(II), Ni(II) and Co(II) complexes on Colon Cancer Cell Line (HT29).

<p>Value *GI50 of <5 μMolar = super active, 5-15 μMolar = Moderately active, 15-40 μMolar = Weakly active , 31-100 μMolar = Resistantant, >100 μMolar = inactive</p>	<p>GI50 = Concentration of drug causing 50% inhibition of cell growth TGI = Concentration of drug causing total inhibition of cell growth ADR = Adriamycin, Positive</p>
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Estimations based on GI50 values shows that ligand, and its Mn(II), Co(II) and Ni(II) complexes were more active against MCF7 than cell HT29 line. The observed order of anticancer activity for series of metal complexes is $\text{Mn} < \text{Co} < \text{Ni}$ [34]. Cytotoxicity of Schiff base (HL) and its Mn(II), Ni(II) and Co(II) complexes on human Breast cancer cell line (MCF7) and Colon Cancer Cell Line (HT29) Shown in figure 4 and figure 5 respectively.

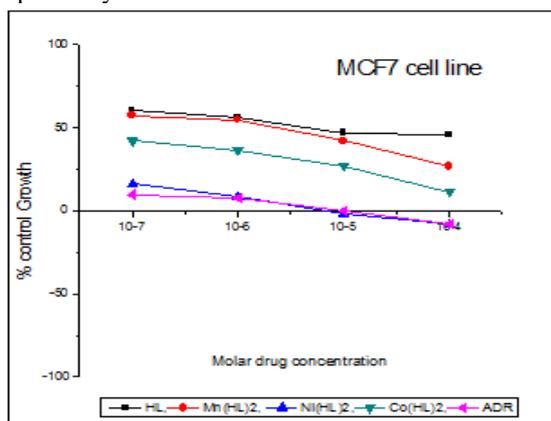


Figure 4. Cytotoxicity of Schiff base (HL) and its Mn(II), Ni(II) and Co(II) complexes human Breast Cancer Cell Line (MCF7)

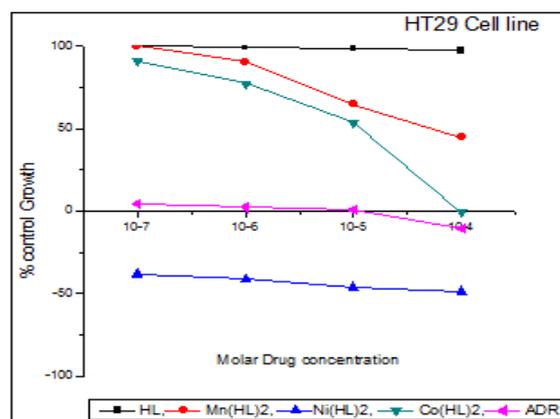


Figure 4. Cytotoxicity of Schiff base (HL) and its Mn(II), Ni(II) and Co(II) complexes on Colon Cancer Cell Line (HT29).

The antidiabetic activities of the synthesized compounds were evaluated *in vitro* by amylase inhibition assay using DNSA method [35-36]. The results of the Antidiabetic studies ligand (HL) and its Mn(II), Co(II) and Ni(II) complexes are given in Table 7.

Compounds	Conc. (µg)	OD at 540 nm	Conc.of Maltose liberated (µg)	Activity (µmoles/ml/min)	% Activity	% Inhibition
Control	Control	1.91	133.33	0.0370	100.00	0.00
[Ni(HL) ₂]	(100 µg)	2.01	141.67	0.0393	106.26	- 6.26
	(500 µg)	2.16	154.17	0.0428	115.64	-15.64
	(1000 µg)	1.82	125.83	0.0349	94.39	5.61
[Mn(HL) ₂]	(100 µg)	2.02	142.31	0.0395	107	-7
	(500 µg)	2.20	155.61	0.0432	117	-17
	(1000 µg)	1.85	127.00	0.0355	96	4.00
[Co(HL) ₂]	(100 µg)	1.97	138	0.0384	104	- 4
	(500 µg)	2.10	149	0.0414	112	- 12
	(1000 µg)	1.90	131.33	0.0364	98.5	1.5

Table 9: Alpha amylase inhibitory activity of metal complexes at various concentrations.

Metal complexes in general have exhibited better antidiabetic activity than ligand. The Ni complex has shown 5.61% inhibition of amylase at 1000 µg, while other sample has induced the amylase activity. Alpha amylase inhibitory activity of Ni(II) complex shown in Figure 6.

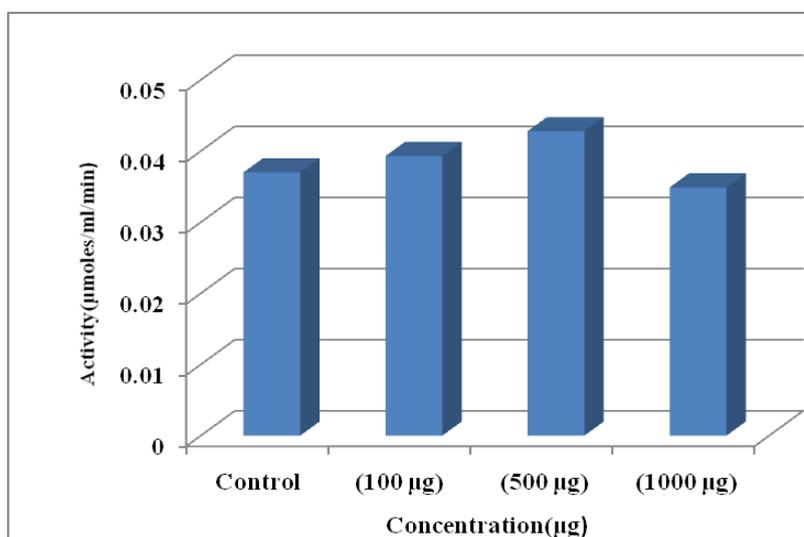


Figure 6. Alpha amylase inhibitory activity of Ni (II) complex at various concentration.

All the complexes have octahedral geometry Nickel complex shows more antimicrobial activity than Cobalt and Manganese complexes. All these complexes showed significant anticancer activity, the Nickel complex showing potency GI50 values of < 0.1 µM for both MCF7 than HT29 cell line which comparable to positive control compound Adriamycin with IC50 of < 0.1 µM. Estimations based on GI50 values shows that ligand and its Mn(II), Co(II) and Ni(II) complexes were active against MCF7 than HT29 cell line. Antidiabetic activity of the Ni(II) complex has shown 5.61% inhibition of amylase at 1000 µg, while other sample has induced alpha amylase activity. The results are very interesting and much useful for drug designing.

III. ACKNOWLEDGEMENT

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