How to Cite: Sarwade, K.N., Sakhare, K.B., Sakhare, M.A., & Thakur, Sh.V. (2025). Neuroprotective, Anticancer and Antimicrobial Activities of Azo-Schiff Base Ligand and Its Metal Complexes. *Eurasian Journal of Chemistry, 30, 1(117), 4–14.* https://doi.org/10.31489/2959-0663/1-25-8

Article

Received: 23 December 2024 | Revised: 17 February 2025 |
Accepted: 21 February 2025 | Published online: 13 March 2025

UDC 547.5, 541.49

https://doi.org/10.31489/2959-0663/1-25-8

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Neuroprotective, Anticancer and Antimicrobial Activities of Azo-Schiff Base Ligand and Its Metal Complexes

The synthesis of metal complexes of Mn(II), Co(II), Ni(II), Cu(II) Zn(II) and VO(II) from azo-Schiff base ligand was carried out by the condensation reaction of (*E*)-5-chloro-2-hydroxy-3-((3-nitrophenyl)diazenyl) benzaldehyde and 2-amino-4-nitrophenol. The structure of the synthesized azo-Schiff base ligand was confirmed by 1H-NMR spectra, mass spectra, FT-IR spectra. After confirmation of the azo-Schiff base ligand, metal complexes of Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and VO(II) were synthesized using metal salts. These synthesized metal complexes were characterized by FT-IR spectra, elemental analysis, electronic spectra, thermal analysis, X-ray powder diffraction, molar conductivity etc. In biological studies, the neuroprotective and anticancer activities of azo-Schiff base ligand and metal complexes were investigated by methylthiazole tetrazolium (MTT) assay on SHSY-5Y Neuroblastoma Cell line and MCF-7 human breast cancer cell line using Tacrine (TAC) and 5-Flourouracil (5-FU) as reference standard drugs, respectively. Also, antimicrobial potential was screened against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*), two gram-negative (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) bacteria and three fungi (*Penicillium chrysogenum*, *Tricoderma viride*, and *Aspergillus niger*). Based on all the data obtained, it was concluded that metal complexes exhibit higher biological activity than ligands based on the azo-Schiff base.

Keywords: azo-Schiff base ligand, coordination compounds, metal complexes, azomethine, biological activity, neuroprotective, anticancer, antimicrobial.

Introduction

Breast cancer is the most common type of cancer and the second leading cause of death for women globally. We lose almost 50000 women worldwide each year due to the breast cancer. Designing effective drugs to manage drug resistance and its side effects is a challenge [1, 2]. The progressive neurodegenerative brain disorder known as Alzheimer's disease that cause neuronal death in the brain and it causes dementia, memory loss, cognitive impairment, severe behavioural abnormalities and finally death. Alzheimer's disease affects around 40 million peoples now and is predicted to reach 150 million by 2050. Many scientists from the world are busy to find effective solution on the breast cancer as well as Alzheimer's disease [3–5]. The Schiff base ligands are extensively used in the metal-ligand chemistry, medicinal chemistry, organic synthesis as catalyst [6]. Schiff bases exhibit different biological activities such as anticancer, antimycobacterial, antibacterial, antidepressant, antianalgesic, antidiabetic, antiviral, anti-infective [7–12]. The Schiff base is condensation product of primary amine with aldehyde. This reaction was firstly studied by noble prize winner, German scientist chemist Hugo Schiff in 1864 [13]. Metal complexes of Schiff base ligands have attracted to researcher because of biological activity in addition to their applications and spectroscopic characteristics and also the presence of donor atoms including nitrogen, oxygen and sulphur [14]. They are used as medications to treat cardiovascular disease, diabetes, cancer and inflammation [15]. According to the literature review, derivatives of salicylaldehyde with one or more halogen atoms in the aromatic ring exhibited a variety of biological activities including antifungal, antibacterial and antimicrobial properties [16].

In the present study, we have focused on the synthesis of novel azo halogenated salicylaldehyde and their Schiff base ligand with 2-amino-4-nitrophenol and its metal complexes.

Experimental

Materials and Methods

Spectrochem supplied all compounds used at analytical grade. The solvents which included petroleum ether, ethanol, ethyl acetate and n-hexane were obtained from a nearby supplier and purified via distillation. The progress of reactions was tracked using thin-layer chromatography utilizing pre-coated aluminium sheets. Mass spectra were captured using a MALDI-TOF mass spectrometer. ^{1}H-NMR spectra were recorded on BRUKER 400MHz ^{1}H-NMR-spectrometer in DMSO, TMS was utilized as the internal standard. Infrared spectra were recorded with FTIR-spectrophotometer (SHIMADZU) and KBr pellets encompassing the spectral range from 4000 to 400 cm^{-1}. Electronic spectra were scanned using SHIMADZU Model 1800 UV-spectrophotometer in DMSO encompassing at room temperature the wavelength range of 200 to 600 nm. A digital conductivity meter model 304 was used to measure molar conductivity in a DMSO solvent. Using TGA instruments the complexes were subjected to a nitrogen environment for thermal analysis.

Biological Activity

Neuroprotective Activity and Anticancer Activity by MTT Assay

The Neuroprotective and Anticancer activity of azo-Schiff base ligand and its metal complexes was carried out using 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay on the SHSY-5Y Neuroblastoma Cell line and MCF-7 human breast cancer cell line respectively. 1×10^4 cells/ml in culture medium was incubated for 24 hours at 37 °C with 5 % CO_2. The cell line was seeded at 1×10^4 cells/well in 100 µl of culture medium at a concentration of 70 µl. 100 µl of the sample was placed into each micro plate. In control wells DMSO (0.2 % in PBS) and cell lines were grown. Every sample was incubated for three rounds. In order to determine the percentage of living cells following cultivation and the survival rate of control cells by controls were maintained. In a CO_2 incubator with 5 % CO_2 cell cultures were maintained at 37 °C for 24 hours. After the incubation period 20 µl of MTT reagent was applied and the media was completely removed. Cells were cultivated for four hours at 37 °C in a CO_2 incubator following MTT injection used a microscope to look for formazan crystal development in the wells. Only living cells were able to transform the yellowish MTT into a dark-coloured formazan. After removing the medium, 200 µl DMSO was added and the mixture was left at 37 °C. Using an Elisa microplate reader set to 570 nm in wavelength 3 samples absorbance were measured in order to conduct analysis. The percent of inhibition and cell viability is calculated by formula given below:

$$\text{Inhibition percentage} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100\,;$$

$$\text{Viability percentage} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100\,.$$

Antimicrobial activity

The National Committee for Clinical Laboratory Standards (NCCLS) 2002 standards were followed for conducting the antibacterial activity utilizing the agar disk diffusion method. To begin, Petri dishes were prepared using MHA (Mueller-Hinton) for bacterial cultures and PDA (Potato Dextrose) for yeast cultures. Preparation of McFarland 0.5 turbidity standards by using the solution of 0.5 ml $BaCl_2\cdot2H_2O$ (Barium Chloride Dihydrate) + 99.5 ml of H_2SO_4 with constant stirring to maintain the suspension and stored in a dark room at 22 to 25 °C. Following the incubation, the application of azo-Schiff base ligand and the metal complexes was made to agar plates that had been previously inoculated. Each plate underwent a visual examination. The resulting zones of inhibition displayed a uniform circular pattern within the growth lawn. The diameters of these inhibition zones along with the diameter of the well in millimetres were measured using sliding callipers and recorded.

Synthesis of Azo-Halogenated Salicylaldehyde

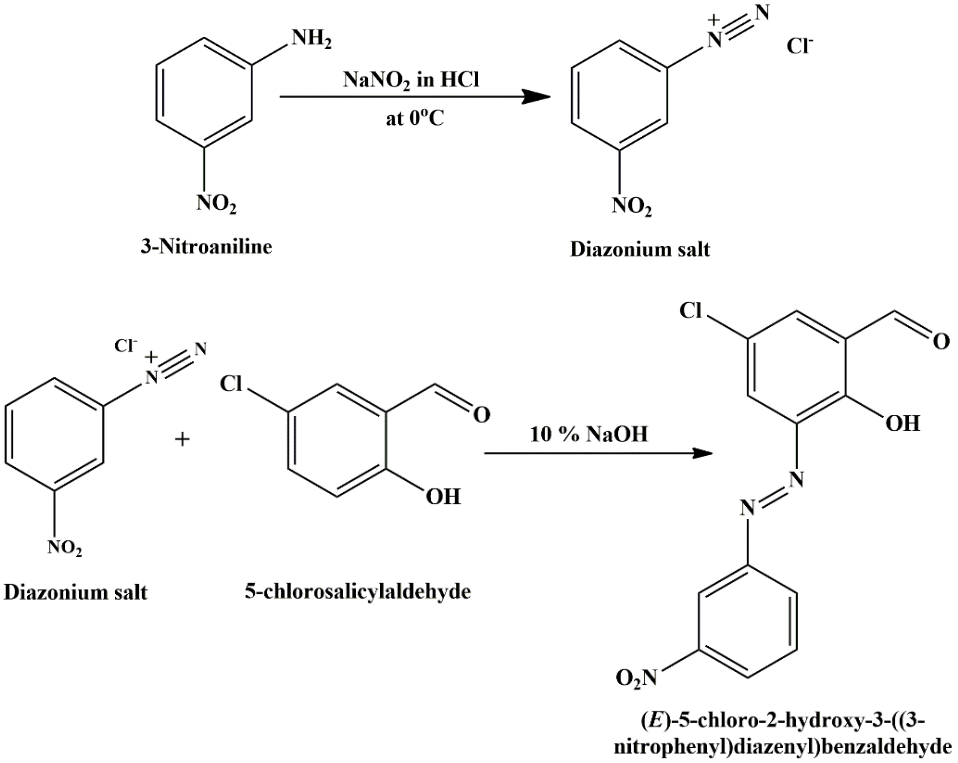
The Azo halogenated salicylaldehyde named (*E*)-5-chloro-2-hydroxy-3-((3-nitrophenyl)diazenyl) benzaldehyde has been synthesized by the diazotization coupling reaction [17].

Diazonium Salt Formation

Solution (a): In 100 ml beaker 5.138 g (0.0372 mol) of 3-nitroaniline, added 25 ml concentrated hydrochloric acid and 20 ml cold distilled water. The solution was cooled to 0 °C using an ice-salt bath.

Solution (b): In a 100 ml beaker 3.105 g (0.045 mol) of sodium nitrite dissolved in 15 ml distilled water and the solution cooled to 0 °C using an ice-salt bath. Solution (b) was added dropwise to a solution (a) while stirring continuously once both solutions (a) and (b) had attained 0 °C. During the addition, the reaction temperature was maintained below 10 °C. A pinch of urea was added to decompose the excess nitrous acid and filtered the diazonium salt solution.

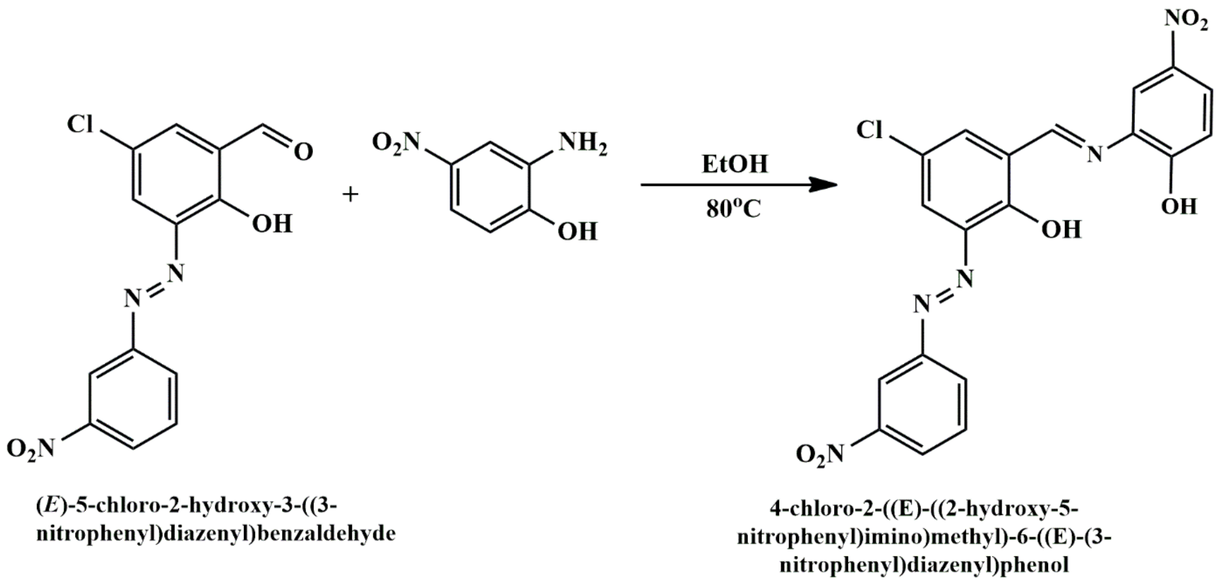
Azo-coupling reaction: 5.824 g (0.0372 mol) of 5-Chlorosalicylaldehyde dissolved in 10 % of NaOH solution and stirred to make the clear solution, cooled the solution to 0 °C in ice-salt bath. Then the above solution of diazonium salt was added to this solution of salicylaldehyde with constant stirring. The reaction mixture was stirred in ice-salt bath for ten minutes after the addition was finished. After obtaining the product with a golden yellow colour, it was filtered, cleaned with cold distilled water, recrystallized and kept in an airtight container (Scheme 1).



Scheme 1. Synthesis of Azo-halogenated salicylaldehyde

General Procedure of Synthesis of Azo-Schiff Base Ligand (L)

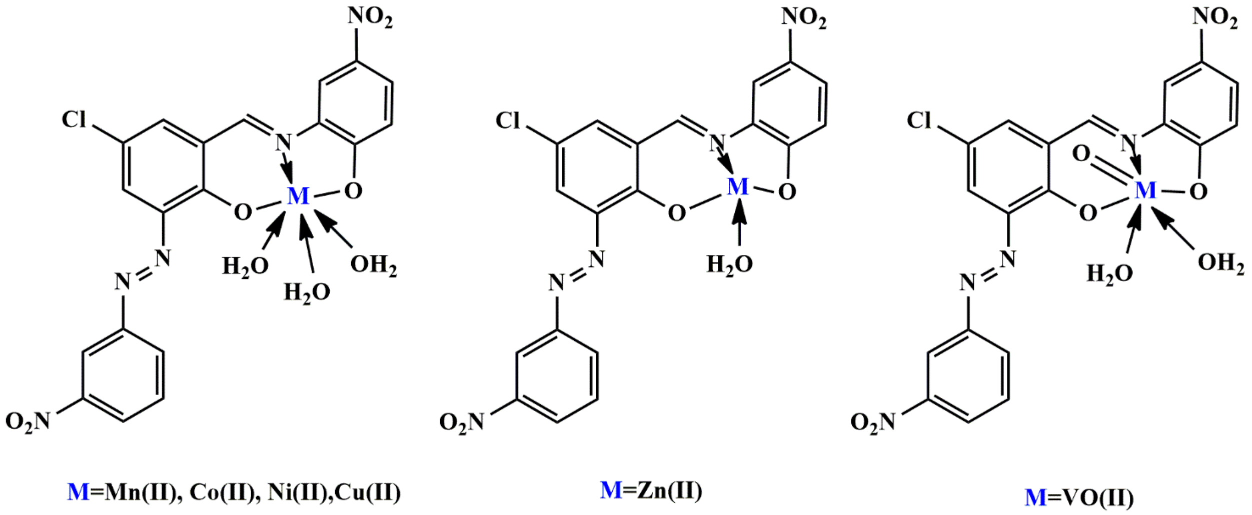
The azo-Schiff base ligand was synthesized by using 3.050 g (0.01 mol) of (*E*)-5-chloro-2-hydroxy-3-((3-nitrophenyl) diazenyl) benzaldehyde dissolved in 25 ml of hot ethanol and 1.541 g (0.01 mol) of 2-amino-4-nitrophenol dissolved in 15 ml of hot ethanol. These two ethanolic solutions were mixed and then refluxed with a temperature range of 70–80 °C for duration of 60 minutes. The reaction's progress was continuously monitored by thin-layer chromatography (TLC). The reaction mixture yielded a solid product with an orange precipitation at room temperature. This solid product was isolated by filtration and recrystallized using ethanol. The product was dried in vacuum over anhydrous $CaCl_2$ overnight to give analytically pure product in good yields [17, 18].



Scheme 2. Synthesis of Azo-Schiff base ligand

General Procedure of Synthesis of Metal Complexes (M)

0.441 g (0.001mol) of azo-Schiff base ligand (L) was dissolved in 15 ml of ethanol. Simultaneously, 0.001 mole of metal acetate salts, including Mn(II), Co(II), Ni(II), Cu(II), Zn(II) metals and the sulphate of VO(II) were dissolved in 15 ml of ethanol. These two ethanolic solutions were mixed and refluxed for a period of 4 hours within the temperature range of 60–80 °C. The reaction resulted in the formation of various coloured metal complexes in the reaction mixture. These complexes were subsequently isolated by filtration, washed with cold ethanol and then dried under vacuum [19].



Scheme 3. Proposed structure of metal complexes

Results and Discussion

The synthesized azo-Schiff base ligand (L) and its metal complexes (M) display distinct colours. The metal complexes are insoluble in water but soluble in organic solvents such as chloroform, DMSO and DMF. They are stable in an air. Physicochemical data of azo-Schiff base ligand and metal complexes is shown in Table 1.

Table 1

Physiochemical data of azo-Schiff base ligand and metal complexes

No	Compounds	Physical Appearance	Melting Point, °C	Yield, %	Elemental Analysis Found, % (Calculated in %)		
					C	H	N
1	Azo-salicylaldehyde	Golden yellow	127–132	78	50.99 (51.08)	2.50 (2.64)	12.30 (13.75)
2	Ligand (L)	Orange	160–167	72	51.20 (51.66)	3.04 (2.74)	15.25 (15.85)
3	Mn (II) Complex	Brown	>300	54	46.30 (46.13)	1.52 (2.04)	15.05 (14.16)
4	Co (II) Complex	Dark Brown	>300	65	41.52 (41.29)	3.58 (2.92)	13.08 (12.67)
5	Ni (II) Complex	Brown	>300	51	41.21 (41.30)	3.13 (2.92)	13.11 (12.68)
6	Cu (II) Complex	Green	>300	73	40.16 (40.94)	2.78 (2.89)	12.02 (12.57)
7	Zn (II) Complex	Yellow	>300	68	43.96 (43.62)	2.00 (2.31)	12.92 (13.39)
8	VO (II) Complex	Green	>300	55	43.22 (43.49)	2.01 (2.31)	13.96 (13.35)

Spectroscopic Analysis

Mass Spectra of Azo Halogenated Salicylaldehyde and Azo-Schiff Base Ligand (L)

The mass spectra in Figures S1, S2 show a peak at m/z 306.1(M+1) and m/z 442.21(M+1), indicating the formation of azo halogenated salicylaldehyde and azo Schiff base ligand (L), respectively.

^{1}H-NMR Spectra of Azo Halogenated Salicylaldehyde and Azo-Schiff Base Ligand (L)

*Azo halogenated salicylaldehyde (*Fig. S3)*:* ^{1}H NMR (400 MHz, DMSO) δ 10.97 (s, 1H), 10.22 (s, 1H), 8.64–8.22 (m, 1H), 8.20–7.80 (m, 1H), 7.77–7.59 (m, 2H), 7.12 (dd, *J* = 8.6 Hz, 2H) [20].

Azo-Schiff base ligand (L) (Fig. S4)*:* ^{1}H NMR (400 MHz, DMSO) δ 13.12 (s, 1H), 10.25 (s, 1H), 9.11 (s, 1H), 8.30 (d, *J* = 2.7 Hz, 1H), 8.12 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.85 (d, *J* = 2.7 Hz, 1H), 7.57 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.52–7.43 (m, 2H), 7.41 (dd, *J* = 8.7, 2.8 Hz, 1H), 7.15 (d, *J* = 9.0 Hz, 1H), 7.04 (d, *J* = 3.8 Hz, 1H) [21].

IR Spectra of L and Metal Complexes

The IR spectra of the azo-Schiff base ligands and metal complexes are shown in Figures S5–S11 and listed in Table 2. In the IR spectra of azo-Schiff base ligand band appeared at 1595 cm^{-1} which shifted to lower frequencies in the range of 1571–1577 cm^{-1} in all the metal complexes. This change implies that the metal ion and the nitrogen of the Schiff base enter into coordination, resulting in the formation of metal complexes [22]. The phenolic -OH group was identified by a large band in ligand located at 3195 cm^{-1} and 3108 cm^{-1}. Interestingly, this band vanished in every metal complex indicating that the phenolic oxygen had coordinated by deprotonation. This observation is further supported by the IR spectra of ligand where a >C–O band at 1232 cm^{-1} shifted to higher frequencies range of 1255–1273 cm^{-1} in all the metal complexes [23, 24]. The azo group's band was originally observed at 1384 cm^{-1}. New bands in the range of 441–447 cm^{-1} and 518–528 cm^{-1} respectively appeared in the metal complexes and were attributed to M–N and M–O vibrations [20]. These findings suggest the formation of coordination bonds among the metal ions and ligand leading to changes in IR spectra which provide insights into the chelation process. The band of >C–Cl exhibited at 642–650 cm^{-1} in ligand and metal complexes. A band of –OH rocking at 821–837 cm^{-1} suggests the presence of coordinated water in all metal complexes [25]. The azo-Schiff base coupled to the metal ion in a tridentate manner according to the overall infrared data.

Table 2

FTIR stretching frequency of azo-Schiff base ligand and metal complexes in cm^{-1}

Sr. No	Compounds	OH– (Phenolic)	>C=N– (Azomethine)	–N=N–	>C–O	>C–Cl	M–N	M–O
1	Ligand (L)	3108, 3195	1595	1384	1232	642	–	–
2	Mn (II)	–	1573	1373	1271	648	447	525
3	Co (II)	–	1571	1373	1255	648	447	522
4	Ni (II)	–	1577	1373	1257	650	441	526
5	Cu (II)	–	1575	1373	1257	646	443	518
6	Zn (II)	–	1573	1377	1273	648	439	526
7	VO (II)	–	1575	1375	1271	648	447	528

Electronic Spectra and Magnetic Susceptibility of L and Metal Complexes (M)

The electronic spectra of azo-Schiff base ligand and metal complexes were recorded in DMSO (Dimethylsulfoxide) at ambient temperature between 200–600 nm. The electronic spectra were used to characterize the ligand field surrounding the central metal ion. The electronic spectrum of L contains bands at 290 as well as at 250 nm, due to the $n\rightarrow\pi^*$ and $\pi\rightarrow\pi^*$ transitions. Mn(II) complexes exhibit bands in their electronic spectra at 350 nm which could indicate an octahedral geometry suggested metal to ligand charge transfer transition. The electronic spectra of Co(II) complexes show broad peaks at 420, 370, and 340 nm, which can be tentatively assigned to $^4T_{1g}\rightarrow{}^4T_{2g}$, $^4T_{1g}\rightarrow{}^4T_{1g(P)}$ and $^4T_{1g}\rightarrow{}^4A_{2g}$, respectively. The magnetic moment (μ) value of 3.92 μB suggests that the Co(II) ion is arranged in an octahedral form. The electronic spectra of Ni(II) complexes show bands at 420, 370 and 340 nm which may be tentatively assigned to $^3A_{2g}\rightarrow{}^3T_{2g}$, $^3A_{2g}\rightarrow{}^3T_{1g(f)}$ and $^3A_{2g}\rightarrow{}^3T_{1g(P)}$ correspondingly. The value of the μat 2.98 μB suggests an octahedral geometry around the Ni(II) ion. Cu(II) complexes electronic spectra showed bands at 420 and 350 nm which may be assigned to $^2E_g\rightarrow{}^2T_{2g}$ and ligand to metal charge transitions correspondingly. The value of the magnetic moment at 1.82 μB suggests an distorted octahedral geometry around Cu(II) ion. Electronic spectra of Zn(II) complexes at 360 nm,, which can mediate charge transfer from the metal to the ligand, show a diamagnetic moment suggesting tetrahedral geometry. The VO(II) complex shows bands at 440, 390 and 325 nm, which may be attributed to the $^2B_2\rightarrow{}^2E$, $^2B_2\rightarrow{}^2B_1$ and $^2B_2\rightarrow{}^2A_1$ transfers. The value of the magnetic moment is 1.79 μB, which is characteristic of the geometry of a square pyramidal structure [26–28]. The absorption band is shown in Figure S12.

Measurement of Molar Conductance of L and Metal Complexes (M)

The measured molar conductance values for L and the metal complexes in a 10^{-3} M dimethylsulfoxide (DMSO) solution at room temperature ranged between 8.5 to 13 $S\cdot mol^{-1}\cdot cm^2$. The relatively low conductance values listed in Table 3 clearly indicate that both L and the metal complexes are non-electrolytic in nature [29].

Table 3

Magnetic moment and molar conductance of azo-Schiff base ligand and metal complexes

Sr. No.	Compounds	Molar conductance in $S\cdot mol^{-1}cm^2$	Magnetic Moment in μB	Proposed Structure
1	Ligand (L)	–	–	–
2	Mn (II) Complex	9.2	5.12	Octahedral
3	Co (II) Complex	8.5	3.92	Octahedral
4	Ni (II) Complex	10	2.98	Octahedral
5	Cu (II) Complex	13	1.82	Octahedral
6	Zn (II) Complex	8.5	Dia.	Tetrahedral
7	VO (II) Complex	12	1.79	Square Pyramidal

Thermal Analysis of Metal Complexes (M)

The thermal stability of the metal complexes synthesized from azo-Schiff base ligand was evaluated under a nitrogen atmosphere covering a temperature range from ambient temperature to 800 °C. The decomposition of the metal complexes took place in two distinct steps. In the first step, the loss of coordinated water molecule takes place up to 250 °C in all metal complexes. Finally, the ligand loss occurred in the temperature range of 300–500 °C and at last metal oxides were formed above 500 °C [25]. The graph observed from the data is given in Figure S13.

Powder X-Ray Diffraction of Metal Complexes (M)

The P-XRD of metal complexes derived from ligand were scanned in the range from $2\theta = 20\text{–}80°$ at wavelength of 1.540 Å. This is given in Table 4 and Figure S14. The Mn(II) and Co(II) complex shows orthorhombic crystal system. The metal complexes Ni(II), VO(II) show monoclinic and Cu(II), Zn(II) show the triclinic crystal system [24].

Table 4

P-XRD metalcomplexes

Sr. No.	Compounds	No. of reflections	Maxima (2θ)	d-Value	Lattice Constant(Å)	Unit cell volume	Axis and Axis angle	Z-Value	Crystal System
1.	Mn(II) Complex	17	9.34	9.462	a = 7.3928 b = 10.4467 c = 18.9250	1461.585	$a \neq b \neq c$ and $\alpha = \beta = \gamma = 90°$	8	Orthorhombic
2.	Co(II) Complex	14	10.39	8.509	a = 4.7487 b = 5.9711 c = 17.0170	482.516	$a \neq b \neq c$ and $\alpha = \beta = \gamma = 90°$	4	Orthorhombic
3.	Ni(II) Complex	13	11.32	7.807	a = 7.8473 b = 7.7271 c = 9.2429	557.614	$a \neq b \neq c$ and $\alpha = \gamma = 90° \neq \beta$	4	Monoclinic
4.	Cu(II) Complex	11	6.81	12.976	a = 9.4450 b = 10.3974 c = 13.3661	1173.400	$a \neq b \neq c$ and $\alpha \neq \beta \neq \gamma \neq 90°$	2	Triclinic
5.	Zn(II) Complex	14	5.31	16.627	a = 4.3633 b = 9.2982 c = 16.7339	662.568	$a \neq b \neq c$ and $\alpha \neq \beta \neq \gamma \neq 90°$	2	Triclinic
6.	VO(II) Complex	23	26.36	12.041	a = 25.5512 b = 7.5077 c = 17.0818	3088.439	$a \neq b \neq c$ and $\alpha = \gamma = 90° \neq \beta$	8	Monoclinic

Biological Activity of L and Metal Complexes

Neuroprotective Activity

The Neuroprotective activity of L and metal complexes was conducted using the MTT assay on the SHSY-5Y Neuroblastoma cell line at different concentration. The results are shown in Table 5.

Table 5

Anti-Alzheimer activity of azo-Schiff base ligand and metal complexes

Sr. No	Compounds	Concentration of Sample (μg/ml)	Cell Viability in %	IC50
1	Standard TAC	25	70.20	49.01
		50	77.41	
		100	78.68	
2	L	25	46.92	78.51
		50	47.92	
		100	47.89	
3	Co(II) Complex	25	39.82	93.28
		50	39.68	
		100	40.55	
4	Cu(II) Complex	25	70.01	50.59
		50	73.59	
		100	75.39	
5	Zn(II) Complex	25	70.59	52.28
		50	70.17	
		100	72.78	

Based on the data presented in Table 5, it can be concluded that Cu(II) and Zn(II) complexes exhibit higher neuroprotection than the ligand and the Co(II) complex compared to the standard reference drug tacrine. The IC50 of Cu(II) and Zn(II) complexes is 50.59 and 52.28 μg/ml. These metal complexes are non-toxic to SHSY-5Y Neuroblastoma cell line. In neurodegenerative disorders, the Schiff base ligand Cu(II) and Zn(II) complexes are frequently dysregulated. Greater cell viability is preferable in the neuroprotective disorders as it shields to the SHSY-5Y Neuroblastoma Cell line from harm which is ideal for neuroprotection [30–33].

Anticancer Activity

The anticancer activity for both L and the metal complexes was examined using the MTT assay on the MCF-7 human breast cancer cell line at different concentration. The results are shown in Table 6.

Table 6

Anticancer activity of azo-Schiff base ligand and metal complexes

Sr. No	Compounds	Concentration of Sample (µg/ml)	Cell Inhibition in %	IC50
1	Standard 5-FU	10	79.14	44.99
		40	83.29	
		100	88.79	
2	L	10	24.38	102.56
		40	37.06	
		100	39.78	
3	Co(II) Complex	10	69.20	51.22
		40	71.40	
		100	77.72	
4	Cu(II) Complex	10	38.79	84.44
		40	43.62	
		100	47.95	
5	Zn(II) Complex	10	46.47	76.75
		40	50.60	
		100	51.53	

As can be seen in Table 6, the Co(II) complex should have anticancer components that are more active in inhibiting the growth of MCF-7 breast cancer cell line compared to other metal complexes. The Co(II) complex demonstrated good anticancer activity compared to L against standard drug 5-FU [34, 35].

Antimicrobial Activity

In our investigation, we evaluated the antibacterial activities of L and its metal complexes against gram-positive bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis* as well as gram-negative bacteria i.e. *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. We also examined their antifungal activity against *Penicillium chrysogenum*, *Tricoderma viride,* and *Aspergillus niger*. The results are shown in Table 7.

Table 7

Antimicrobial activity of azo-Schiff base ligand and metal complexes (Zone of inhibition in mm)

		Antibacterial activity				Antifungal activity		
Sr. No.	Compounds	*S. aureus*	*B. subtilis*	*K. pneumoniae*	*P. aeruginosa*	*P. Chrysogenum*	*T. Viride*	*A. Niger*
1	Ligand (L)	11	14	10	8	8	8	12
2	Mn (II)	16.5	23.5	19.5	8	15.5	30.5	24
3	Co (II)	18	21	16	18.5	19.5	27	21.5
4	Ni (II)	17.5	13	19.5	14	20.5	29.5	8
5	Cu (II)	18	19	15	16.5	24.5	24.5	8
6	Zn (II)	16.5	20	14	12.5	8	20.5	19
7	VO(II)	10	15	13	16	12	15	12
8	Tetracycline	19	25	20	19	–	–	–
9	Fluconazole	–	–	–	–	25	35	26

The results of antibacterial screening in Table 7 showed that Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) metal complexes exhibit greater activity than ligand but less than that of standard reference drug tetracycline. In the antifungal screening the Mn(II), Co(II), Ni(II) and Zn(II) complexes exhibited a higher activity and

Cu(II) and VO(II) complexes demonstrated moderate level of activity than ligand in comparison to the standard reference drug fluconazole [36, 37].

Conclusions

In this paper, we presented the synthesis and characterization of novel azo-Schiff base ligand and its transition metal complexes. From the findings of various spectrochemical techniques, we confirmed that ligand show tridentate behaviour, forming bonds with metal through the azomethine nitrogen and phenolic oxygen atoms. The P-XRD analysis of the complexes revealed a variety of crystal systems including orthorhombic, monoclinic and triclinic structures. The Cu(II) and Zn(II) complexes exhibited greater neuroprotective activity and Co(II) complex shows better anticancer activity than azo-Schiff base ligand. The metal complexes exhibit higher antimicrobial activity than azo-Schiff base ligand. The azo-Schiff base ligand is active, but if metal complexes are made by coordinating various metal salts the activities increase.

Supporting Information

The Supporting Information is available free at https://ejc.buketov.edu.kz/index.php/ejc/article/view/222/182

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Kirti Navnath Sarwade** investigation, methodology; ***Shailendrasingh Virendrasingh Thakur*** conceptualization, visualization; **Kuldeep Bhaskar Sakhare** writing-original draft, formal analysis; **Mahadeo Abarao Sakhare** validation, writing-review & editing.

Acknowledgments

The authors are thankful to the Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for providing the facility of FTIR and UV Spectroscopy, Principal Shri Shivaji Science College Amaravati for providing XRD analysis, Director, Biocyte institute of research and development, Sangli for biological activities, Authors also wish to extend their gratitude to the Principal, Balbhim Arts, Science and Commerce College Beed for providing necessary laboratory facility.

Conflicts of Interest

The authors declare no conflict of interest, financial or otherwise.

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