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# Synthesis of metal-based biologically active agents from ONO-donor Schiff base ligand

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## ABSTRACT

The coordination complexes of Mn(II), Co(II), Ni(II), Cu(II) Zn(II) and VO(II) derived from novel azo-Schiff base ligand 2-((E)-(5-bromo-2-hydroxy-3-((E)-((3-hydroxypyridin-2-yl)imino)methyl)phenyl)diazenyl)pyridin-3-ol using (E)-5-bromo-2-hydroxy-3-((3-hydroxy-pyridin-2-yl)diazenyl)benzaldehyde with 2-amino-3-hydroxy pyridine. These are characterized by FT-IR spectra, 1H-NMR spectra, mass spectra, electronic spectra, elemental analysis, thermal analysis, X-ray powder diffraction, molar conductivity etc. In biological studies, the synthesized ligand and its metal complexes were screened for antimicrobial activity against two-gram positive bacteria, two-gram negative bacteria and three fungi. The neuroprotective activity was tested against SHSY-5Y Neuroblastoma cell line by MTT assay. The anticancer activity was screened against the human MCF-7 breast cancer cell line by using Sulfo-Rhodamine-B-stain (SRB) assay. The observations suggest that metal complexes exhibited good activities than ligand.

**Keywords:** Azo-Schiff base, 5-Bromosalicylaldehyde, SHSY-5Y cell, 2-amino-3-hydroxy pyridine, MCF-7

## INTRODUCTION

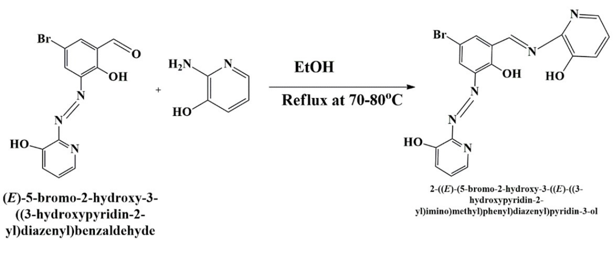
The azomethine nitrogen (>C=N-) of Schiff base ligands are focused as they play vital role in medicinal chemistry due to their broad spectrum of biological as well as biochemical activities. The Schiff base ligand synthesized from condensation of aldehyde and amine has shown anti-Alzheimer, anticancer, antifungal and antibacterial effect as biological important molecules [1]. Derivatives of 2-aminophenol and 2-amino-3-hydroxy pyridine holds biological significance in the synthesis of clinical anti-inflammatory, analgesic drugs and their Schiff base ligand shows anti-Alzheimer activity [2, 3]. The most prevalent cause of dementia known as Alzheimer is characterized by gradual cognitive impairment in the early stages of life, memory loss, and several behavioural abnormalities. It affects up to 10% of the population over 65 ages and is diagnosed after the age of 56. The disease Alzheimer is related to the age. 30% of people over 80 ages are afflicted by the Alzheimer. In the developed world, Alzheimer disease comes in fourth place after cardiovascular disease, cancer, and cerebrovascular accidents as the leading causes of death. There are currently 40 million peoples suffering from the Alzheimer disease globally and by 2050 that figure is predicted to rise up-to 150 million [4-7]. Current treatments for Alzheimer disease aim to stop or slower the progression of disease. Inventing more effective methods of treatment have become a global effort, as the present treatments for Alzheimer disease is not curative. Many scientists form the world are busy to find effective solution on the disease Alzheimer. The heterocyclic molecule containing nitrogen and oxygen atoms have various biological activities. Isatin based, isovanillin based, 4-aminoantipyrine based Schiff base ligand exhibits anti-Alzheimer, anticancer, antioxidant activity. Schiff base ligands that incorporate with halogen groups and their corresponding metal complexes have become part of particular interest of researcher due to their antimicrobial properties [8-15]. Recently, increased studies on the anti-Alzheimer, anticancer activity encouraged us to carry out this work. In this work, we have represented the synthesis of novel azo-Schiff base ligand by condensation of 2-amino-3-hydroxy pyridine with derivative of salicylaldehyde and its transition metal complexes by utilising various transition metal salts. Another important goal of this study is to examine the anti-Alzheimer, anticancer activity and antimicrobial activities of these compounds.

## EXPERIMENTAL

***Materials and methods:*** All the chemicals utilized were of AR (analytical grade) and were procured from reputed company Spectrochem. The solvents such as ethanol, petroleum ether, ethyl acetate, n-hexane were purchased from local provider and further purified by distillation. The progression of reactions was tracked using thin-layer chromatography (TLC Plate) which was pre-coated by silica on aluminium sheets. ESI-mass spectrum was captured using a BRUKER Compass Data Analysis 4.2 mass spectrometer. The $^{1}$H-NMR spectra was run in $CDCl_3$ solvent by using a BRUKER 500MHz $^{1}$H-NMR instrument, TMS was utilized as the inbuilt standard. Infrared spectra were recorded with a BRUKER Alpha T spectrophotometer and KBr pellets, encompassing the spectral range from 4000 to 400 $cm^{-1}$. Electronic spectra of ligand and metal complexes were scanned using a PERKIN ELMER UV-spectrophotometer in DMSO, covering the range of wavelength from 200 to 800 nm at room temperature. Parameters of molar conductivity were carried out in a DMSO solvent using a digital conductivity meter of model 304. The thermal analysis of the complexes were conducted under the nitrogen atmosphere, employing the TA Instruments Trios V4.4.0.41128 TG/DSC thermal system.

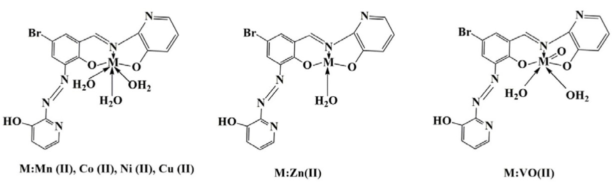
### Synthesis

***General procedure of synthesis of azo-Schiff base ligand:*** The azo-Schiff base ligands were synthesized according to the literature [16]. The azo-Schiff base ligand was prepared by using 3.22 g (0.01 mol) of (E)-5-bromo-2-hydroxy-3-((3-hydroxypyridin-2-yl) diazenyl) benzaldehyde dissolved in 25 ml of hot ethanol and 1.10 g (0.01 mol) of 2-Amino-3-hydroxypyridine dissolved in 15 ml of hot ethanol. The equimolar ethanolic solutions were mixed and then refluxed with a temperature range of 70-80 °C for duration of 2 hours. The progress of reaction was continuously monitored by thin-layer chromatography (TLC). Ultimately, 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.




Scheme 1. Synthesis of azo-Schiff base ligand

***General procedure of synthesis of metal complexes***: The metal complexes were synthesized according to the literature [16-17]. 0.413 g (0.001 mol) of azo-Schiff base ligand was dissolved in 15 ml of ethanol. Simultaneously, 0.001 mol of metal acetate salts, including Mn(II), Co(II), Ni(II), Cu(II), Zn(II) and the sulphate of VO(II) were dissolved in 15 ml of ethanol. These two ethanolic solutions were slowly added to each other and refluxed for a period of 4 hours with the temperature range of 60-80 °C. The reaction mixture 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.




Scheme 2. Proposed structure of metal complexes

***Biological activity***

***Neuroprotective activity:*** The 3-(4,5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay provided the sufficient way for the study of neuroprotective activity of azo-Schiff base ligand and their metal complexes. The SHSY-5Y Neuroblastoma Cell line was procured from National centre for cell sciences (NCCS), Pune maintained in DMEM medium supplemented with 10% fetal bovine serum. The preparation of sample concentration at 25 µg/ml, 50 µg/ml, 100 µg/ml in DMSO. The general method is previously described [18]. The percent of cell viability is calculated by formula given below:

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

***Anticancer activity:*** The Sulfo-Rhodamine-B-Stain SRB assay provided a sufficient way for study of in-vitro cytotoxicity of the azo-Schiff base ligand and metal complexes. The MCF-7 human breast cancer cell line obtained from the National centre for cell sciences (NCCS) Pune maintained in DMEM medium supplemented with 10% fetal bovine serum. It was prepared concentration at 100 µg/mL in DMSO and added to the cancer cell. The general method have previously described [19]. The percent of inhibition is calculated by formula given below:

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

***Antimicrobial activity:*** The antimicrobial activity was assessed against two gram-positive, two gram-negative bacteria and three fungi by disk diffusion method, following the guidelines outlined in the NCCLS (National Committee for Clinical Laboratory Standards) 2002. Utilising standard agar, following literature procedure [15]. DMSO was used as a negative control which showed no inhibition activities against any of the organisms and it was utilized for dissolving all compounds concentration at 100 μg/mL. The zone of inhibition around each disk was measured using sliding callipers in millimetres and comparison with standard reference drug tetracycline and fluconazole as positive control.

## RESULTS AND DISCUSSION

The synthesized azo-Schiff base ligand and its metal complexes display distinct colours. Generally, the metal complexes are insoluble in water but readily soluble in common organic solvents like chloroform and DMF. This solubility behaviour suggests that these complexes may be more suitable for applications in organic solvents rather than aqueous environments. The variation in colours may be indication of different coordination environments or oxidation states of the metal ions in the complexes which can have a significant impact on their properties and reactivity. They are stable in air shown in Table 1.

### Spectroscopic Analysis

***Mass spectra of azo-Schiff base ligand:*** Mass spectrometry is a crucial analytical technique for determining the molecular weight of newly synthesized molecules. In the case of the azo-Schiff base ligand, the molecular ion peak was observed at M/z, which is a significant feature in mass spectrometry for identifying the compound's molecular mass 413.269, 415.0326(M+2) shown in **Fig. S1**.

***$^1$H-NMR spectra of azo-Schiff base ligand:*** The $^1$H-NMR spectra of the azo-Schiff base ligand was recorded in $CDCl_3$ solvent shows in **Fig. S2**. The spectra revealed several significant signals: The signal at δ = 9.41 ppm corresponds to the azo-imine proton (HC=N) [17]. Additionally, peaks observed at δ = 9.84 ppm 10.93 ppm and 12.32 ppm were attributed to the two phenolic-OH groups found within the pyridine moiety and one phenolic-OH groups in the salicylaldehyde moiety respectively. These signals represent the hydroxyl (OH) protons associated with these functional groups. Within the spectra, a multiplet spanning the range of δ = 6.90 ppm to 8.08 ppm was observed, reflecting the presence of protons within the aromatic ring [20].

$^1$H-NMR (500 MHz, $CDCl_3$) δ 12.32 (s, 1H, -OH), 10.93 (s, 1H, -OH), 9.84 (s, 1H, -OH), 9.41 (s, 1H, N=CH), 8.08(d,1H, Ar-H), 7.06 (d, 1H, Ar-H) J=2.5Hz, 6.90-6.96 (dd, 2H, Ar-H),7.65-7.68 (dd, 2H, Ar-H), 7.49-7.52 (dd, 2H, Ar-H).

***FTIR spectra of azo-Schiff base ligand and Metal complexes:*** The IR spectrum of azo-Schiff base ligand and metal complexes illustrated in **Figs. S3-S9** and listed in Table 2.

Table 1. Some physiochemical data of azo-Schiff base ligand and metal complexes

| Compounds | Colour | M.P ($^o$C) | Yield (%) | Elemental analysis found (Calculated) C | H | N |
|---|---|---|---|---|---|---|
| Ligand | Orange | 210 | 87% | 48.84 (49.29) | 3.10 (2.92) | 16.45 (16.91) |
| Mn (II) complex | Maroon | >300 | 52% | 43.15 (43.71) | 2.45 (2.16) | 14.56 (14.99) |
| Co (II) complex | Dark Brown | >300 | 71% | 42.98 (43.34) | 2.45 (2.14) | 14.36 (14.86) |
| Ni (II) complex | Brown | >300 | 59% | 38.01 (38.90) | 2.86 (3.07) | 13.74 (13.34) |
| Cu (II) complex | Green | >300 | 73% | 42.10 (42.92) | 2.58 (2.12) | 14.99 (14.72) |
| Zn (II) complex | Yellow | >300 | 81% | 42.23 (42.75) | 2.44 (2.11) | 14.78 (14.66) |
| VO (II) complex | Dark Green | >300 | 66% | 43.01 (42.61) | 2.48 (2.10) | 14.21 (14.62) |

Table 2. FTIR data of azo-Schiff base ligand and metal complexes

| Frequency in $cm^{-1}$ | | | | | | | |
|---|---|---|---|---|---|---|---|
| Compounds | -OH (Phenolic) | C=N (Azomethine) | -N=N- | >C-Br | >C-O | M-N | M-O |
| Ligand | 3130, 3083 | 1577 | 87% | 662 | 1275 | -- | -- |
| Mn (II) | -- | 1552 | 52% | 651 | 1282 | 451 | 578 |
| Co (II) | -- | 1544 | 71% | 678 | 1298 | 439 | 545 |
| Ni (II) | -- | 1558 | 59% | 678 | 1294 | 460 | 578 |
| Cu (II) | -- | 1556 | 73% | 684 | 1294 | 453 | 582 |
| Zn (II) | -- | 1562 | 81% | 682 | 1290 | 453 | 582 |
| VO (II) | -- | 1440 | 66% | 650 | 1276 | 441 | 597 |

In the FTIR spectra of ligand characteristic band appeared at 1577 $cm^{-1}$ which shifted to lower frequencies within the range 1440 - 1562 $cm^{-1}$ in all the metal complexes. This shift suggests the coordination of the azo-imine nitrogen with the metal ions indicating the formation of metal azo-imine complexes [17]. The IR spectrum of ligand exhibited a broad band at 3130 $cm^{-1}$ and 3083 $cm^{-1}$ which was assigned to the phenolic OH group. Notably, this band disappeared in all the metal complexes signifying the coordination of the phenolic oxygen through deprotonation. This observation is further supported by the IR spectrum of ligand where a band at 1275 $cm^{-1}$ shifted to higher frequencies ranging from 1276-1298 $cm^{-1}$ in all the metal complexes [21-22]. The azo group's band was originally observed at 1357 $cm^{-1}$ in the azo-Schiff base ligand. However, in the metal complexes new bands emerged in the range of 439-460 $cm^{-1}$ and 545-597 $cm^{-1}$ which was assigned to M-N and M-O vibrations respectively. These findings suggest the formation of coordination bonds between the metal ions and the ligand, leading to changes in the IR spectra which provide insights into the chelation process. The band of >C-Br exhibited at 650-684 $cm^{-1}$ in azo Schiff base ligand and metal complexes [23]. A band of -OH rocking at 817-839 $cm^{-1}$ suggests the presence of coordinated water in all metal complexes [24]. The overall IR data of the Schiff bases ligand and its metal complexes showed that the azo-Schiff base coordinated to the metal ion in a tridentate manner.

***Electronic spectra and magnetic susceptibility of azo-Schiff base ligand and Metal complexes:*** The electronic spectra of azo-Schiff base ligand and metal complexes were obtained at ambient temperature in dimethylsulfoxide (DMSO) at 200-600 nm. The electronic spectra was used to determine the nature of the ligand field surrounding the central metal ion. The geometric structures of the synthesized complexes were deduced from magnetic susceptibility measurement. The paramagnetic complexes have affinity to magnetic field while the diamagnetic complexes repelled in magnetic field. In the paramagnetic, the flux is greater within the substance than it would be in vacuum. Therefore, paramagnetic complexes will have positive (+ve) susceptibility. The magnetic study indicates that all metal complexes have paramagnetic character except Zn(II) complex [24, 25]. The electronic spectrum of azo-Schiff base ligand exhibit bands at 249 and 225 nm indicating $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. The electronic spectra of Mn(II) complexes shows bands at 445 nm which may lead to metal to ligand charge transfer transition suggest octahedral geometry. The electronic spectra of Co(II) complexes displayed broad peak at 435, 375 and 260 nm which may be tentatively assigned to $^4T_{1g} \rightarrow {}^4T_{2g}$, $^4T_{1g} \rightarrow {}^4T_{1g(P)}$ and $^4T_{1g} \rightarrow {}^4A_{2g}$ respectively. The value of magnetic moment 4.90 $\mu$B which indicates that octahedral configuration around Co(II) ion. The electronic spectra of Ni(II) complexes shows band at 410, 348 and 270 nm which may $^3A_{2g} \rightarrow {}^3T_{2g}$, $^3A_{2g} \rightarrow {}^3T_{1g(f)}$ and $^3A_{2g} \rightarrow {}^3T_{1g(P)}$ respectively. The value of magnetic moment at 3.1 $\mu$B suggest an octahedral geometry around Ni(II) ion. The electronic spectra of Cu(II) complexes displayed bands at 440 and 400 nm which may $^2E_g \rightarrow {}^2T_{2g}$ and ligand to metal charge transitions respectively. The value of magnetic moment at 1.85 $\mu$B suggest an distorted octahedral geometry around Cu(II) ion due to Jahn-Teller distortion [26]. The electronic spectra of Zn(II) complexes 440 nm which may metal to ligand charge transition and shows diamagnetic moment suggesting tetrahedral geometry. The VO(II) complex shows bands at 500, 450 and 300 nm which may $^2B_2 \rightarrow {}^2E$, $^2B_2 \rightarrow {}^2B_1$ and $^2B_2 \rightarrow {}^2A_1$. The value of magnetic moment at 1.77 $\mu$B which are characteristics of square pyramidal geometry [27]. Absorption band shown in **Figs. S10-S16**. Proposed transition and magnetic moment listed in Table 3.

***Measurement of molar conductance of azo-Schiff base ligand and metal complexes:*** By using the relation, $\Lambda_M$ = K/C, the molar conductance readings for azo-Schiff base ligand and their metal complexes at $10^{-3}$ M dimethylsulfoxide (DMSO) solution at room temperature ranged between 6.4 to 10 $S \cdot mol^{-1} \cdot cm^2$. These relatively low conductance values clear indicates that both ligand and the metal complexes are non-electrolytic in nature. This is accordance with fact that conductivity values for non-electrolytes are below 50 $S \cdot mol^{-1} \cdot cm^2$ in DMSO solution [28]. The listed in Table 3.

***Thermal Analysis of metal complexes:*** The thermal stability of the metal complexes was evaluated under a nitrogen atmosphere spanning a temperature ranging from ambient temperature to 800 °C. The metal complexes decomposed in two distinct steps. In the first step, the loss of coordinated water molecule takes place up to 250 °C in all metal complexes. Lastly, there was a loss of the ligand takes in the range of temp. of 300-500 °C and finally above 500 °C metal oxides are formed [17]. The graph observed from the data are given in **Figs. S17-S22**.

***Powder X-Ray Diffraction of metal complexes:*** The P-XRD of metal complexes formed from ligand was scanned in the range of 2θ = 20 - 80º at 1.540 Å wavelength. This is given in Table 4 and **Figs. S23-28**. The metal complexes Ni(II), Cu(II), Zn(II) and VO(II) shows the tetragonal crystal system and the Mn(II) and Co(II) shows Monoclinic crystal system [21].

**Biological Activity of azo-Schiff base ligand and metal complexes**

***Neuroprotective activity:*** The neuroprotective activity of synthesized azo-Schiff base ligand and their Co(II), Cu(II) and Zn(II) complexes were determined against SHSY-5Y Neuroblastoma Cell line with different concentrations at 25, 50, 100 µg/ml. The present study revealed that the Cu(II) and Zn(II) complex demonstrated the better neuroprotectection with $IC_{50}$

Table 3. Electronic spectra, molar conductance and magnetic moment of azo-Schiff base ligand and metal complexes

| Compounds | $\lambda_{max}$ nm | Absorption band in $cm^{-1}$ | Proposed transitions | Molar conductance (s $mol^{-1}$ $cm^2$) | Magnetic moment (μB) |
|---|---|---|---|---|---|
| Ligand | 249 | 40160 | $n \rightarrow \pi^*$ | 7.1 | -- |
| | 225 | 44444 | $\pi \rightarrow \pi^*$ | | |
| Mn (II) | 445 | 22471 | M → L, CT | 9.7 | 5.19 |
| Co (II) | 435 | 22988 | $^4T_{1g} \rightarrow {}^4T_{2g}$ | 10 | 4.90 |
| | 375 | 26666 | $^4T_{1g} \rightarrow {}^4T_{1g(P)}$ | | |
| | 260 | 38461 | $^4T_{1g} \rightarrow {}^4A_{2g}$ | | |
| Ni (II) | 410 | 24390 | $^3A_{2g} \rightarrow {}^3T_{2g}$ | 6.4 | 3.10 |
| | 348 | 28735 | $^3A_{2g} \rightarrow {}^3T_{1g(f)}$ | | |
| | 270 | 37037 | $^3A_{2g} \rightarrow {}^3T_{1g(P)}$ | | |
| Cu (II) | 440 | 27727 | $^2E_g \rightarrow {}^2T_{2g}$ | 6.7 | 1.85 |
| | 368 | 27173 | L → M, CT | | |
| Zn (II) | 440 | 22727 | M → L, CT | 6.5 | Dia. |
| VO (II) | 500 | 20833 | $^2B_2 \rightarrow {}^2E$ | 6.5 | 1.77 |
| | 450 | 22222 | $^2B_2 \rightarrow {}^2B_1$ | | |
| | 300 | 33333 | $^2B_2$ - $^2A_1$ | | |

Table 4. P-XRD of metal complexes

| Complexes | Mn(II) | Co(II) | Ni(II) | Cu(II) | Zn(II) | VO(II) |
|---|---|---|---|---|---|---|
| No. of reflections | 27 | 10 | 19 | 25 | 29 | 23 |
| Maxima (2θ) | 57.11º | 54.51º | 52.69º | 63.01º | 17.96º | 99.54º |
| Intensity | 82.5 a.u. | 100 a.u. | 76.4 a.u. | 28.1 a.u. | 56.9 a.u. | 100 a.u. |
| d value | 10.508 Å | 14.428 Å | 8.117 Å | 6.168Å | 29.655 Å | 5.226 Å |
| Lattice constant (Å) | a=16.7340<br>b=7.8300<br>c=20.4128 | a=12.2628<br>b=12.867<br>c=15.5143 | a=14.5445<br>b=8.3832<br>c=16.2341 | a=9.0252<br>b=11.0298<br>c=12.3350 | a=59.3100<br>b=25.1410<br>c=31.0500 | a=9.1420<br>b=6.3700<br>c=5.8890 |
| Unit cell volume | 1376.793 | 2276.111 | 1979.415 | 1227.902 | 46299.051 | 342.943 |
| Axis and axis angle | a≠b≠c and α=γ=90º≠β | a≠b≠c and α=γ=90º≠β | a≠b≠c and α=β=γ=90º | a=b≠c and α=β=γ= 90º | a≠b≠c and α=β=γ=90º | a≠b≠c and α=β=γ=90º |
| Z Value | 4 | 2 | 8 | 8 | 4 | 4 |
| Crystal system | Monoclinic | Monoclinic | Tetragonal | Tetragonal | Tetragonal | Tetragonal |

value at 52.24-52.77 μg/ml. The Co(II) complex shows lower neuroprotection with $IC_{50}$ value at 79.74 μg/ml compared with other metal complexes. From the finding we can conclude that Cu(II) and Zn(II) complexes were non-toxic to SHSY-5Y Neuroblastoma Cell line and exhibit significant neuroprotection than ligand in the comparison with standard reference drug tacrine [30-32]. The Cu(II) and Zn(II) complexes of Schiff base ligand are often dysregulated in neurodegenerative diseases. In the neuroprotective activity increased cell viability is better because protecting the SHSY-5Y Neuroblastoma Cell line from damage which is desirable for neuroprotection [18]. The comparative findings are given in Table 5 and Fig. 1.

***Anticancer activity**:* The cytotoxicity of synthesized azo-Schiff base ligand and their Co(II), Cu(II) and Zn(II) complexes were determined against MCF-7 human breast cancer cell line using 5-FU as a reference drug. By comparing the results with those of a previous study [19]. The tested Cu(II) and Zn(II) complexes in the present work exhibited lowest cytotoxicity against the selected human cell lines compared to standard drug 5-FU [15]. But Co(II) complex shows greater cytotoxicity than ligand and slightly better than standard 5-FU. The Co(II) complex should possess anticancerous

Table 5. Anti-Alzheimer activity of L and metal complexes

| Compounds | Concentration (μg/ml) | Cell viability (%) | $IC_{50}$ |
|---|---|---|---|
| Standard TAC | 25 | 70.20 | 48.63 |
| | 50 | 77.41 | |
| | 100 | 78.68 | |
| Ligand | 25 | 56.57 | 61.56 |
| | 50 | 57.90 | |
| | 100 | 63.50 | |
| Co (II) complex | 25 | 40.97 | 79.74 |
| | 50 | 44.69 | |
| | 100 | 49.77 | |
| Cu (II) complex | 25 | 61.39 | 52.77 |
| | 50 | 72.60 | |
| | 100 | 72.71 | |
| Zn (II) complex | 25 | 69.10 | 52.24 |
| | 50 | 70.74 | |
| | 100 | 72.98 | |

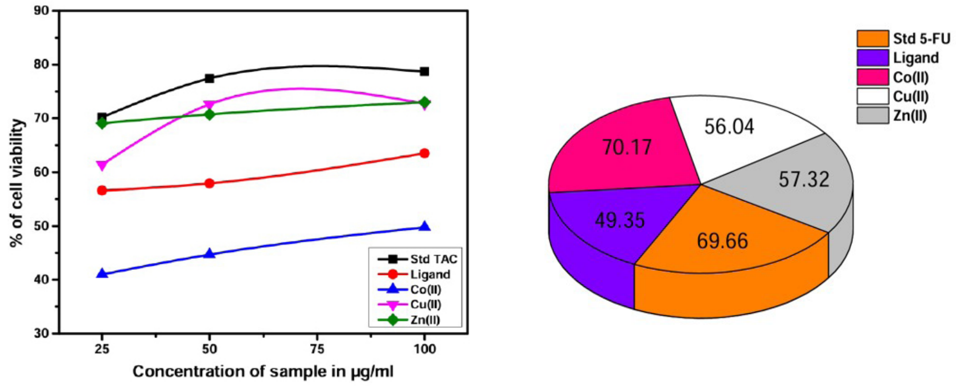



Fig.1. Schematic representation of neuroprotective and anticancer activity

components that inhibit the growth of MCF-7 human cancer cell compared with other metal complexes. The nature of metal ion and the environment of ligand has an impact on the cytotoxicity of complexes. The cytotoxic potency may be explained in that the positive charge of the metal increases the acidity of coordinated ligand that gives proton causing more potent hydrogen bonds which enhance the biological activities. The findings are given in Table 6 and Fig. 1.

Table 6. Anticancer activity of Ligand and metal complexes

| Compounds | Concentration (µg/ml) | Inhibition (%) |
|---|---|---|
| 5-FU (Standard) | 20 | 69.66 |
| Ligand | 100 | 49.35 |
| Co (II) complex | 100 | 70.17 |
| Cu (II) complex | 100 | 56.04 |
| Zn (II) complex | 100 | 57.32 |

***Antimicrobial activity:*** The antibacterial activities of azo-Schiff base ligand and its Mn(II), Co(II), Ni(II), Cu(II) Zn(II), VO(II) complexes by using gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* as well as gram-negative bacteria such as *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

Table 7. Antimicrobial activity of Ligand and metal complexes

| Antibacterial activity | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Zone of inhibition (diameter in mm) | | | | | | | |
| Test Organism | Standard | Ligand | Mn(II) | Co(II) | Ni(II) | Cu(II) | Zn(II) | VO(II) |
| *S. aureus* | 19 | -- | -- | 17 | 8.5 | 16 | -- | 10 |
| *B. subtilis* | 25 | -- | -- | 11.5 | 11 | 10 | -- | 15 |
| *K. pneumoniae* | 20 | 10.5 | -- | 17.5 | 11 | 11.5 | 9 | 17.5 |
| *P. aeruginosa* | 19 | -- | -- | 12.5 | 19 | 13.5 | -- | 18.5 |
| Antifungal activity | | | | | | | | |
| *P.chrysogenum* | 25 | -- | 18 | 18 | 25 | 28 | 19 | 14 |
| *T. viride* | 35 | -- | -- | 15 | 32.2 | 32.2 | 20 | 17.5 |
| *A. niger* | 26 | -- | -- | 10 | 25 | 25 | 10 | -- |

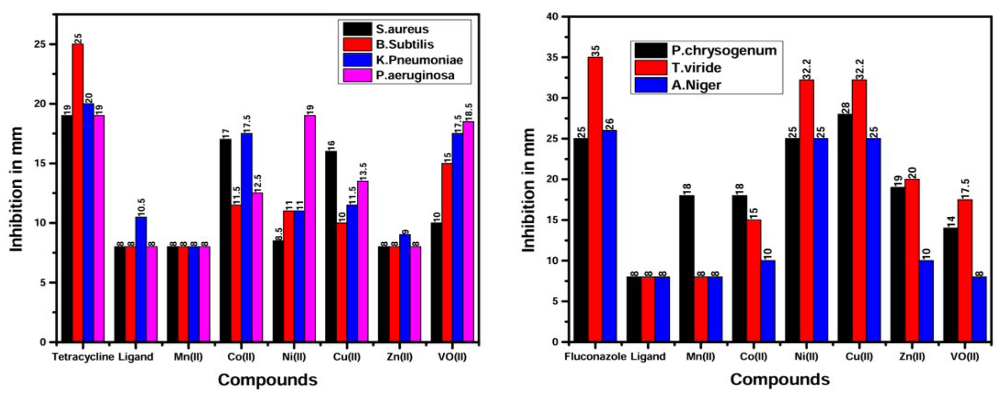



Fig.2. Schematic representation of antimicrobial activity

We also examined their antifungal activity by using fungi such as *Penicillium chrysogenum*, *Tricoderma viride,* and *Aspergillus niger*.The results of antibacterial screening showed that Co(II), Ni(II), Cu(II) and VO(II) metal complexes exhibit greater activity than ligand but less than that of standard reference drug tetracycline. In the antifungal screening the Ni(II), Cu(II) and Zn(II) complexes exhibited a higher activity and Mn(II), Co(II), and VO(II) complexes demonstrated moderate level of activity than ligand in comparison to the standard reference drug fluconazole [33, 34]. According to Tweedy chelation theory, chelation enhances the biological activity of metal complexes and it can be seen that the azo-Schiff base ligand exhibit weak biological activity, while all metal complexes exhibit better activity against bacteria and fungi. This means that the activity azo-Schiff base ligand is enhanced with chelation with various biologically active metals [19] described in Table 7 and the comparative zone of inhibition of ligand and its complexes shown graphically in Fig. 2.

## CONCLUSION

In this paper we have presented the synthesis and characterization of a novel ONO donor Schiff base ligand and its transition metal complexes. Findings of spectral, elemental, and thermal techniques confirmed that ligand have tridentate behaviour, bonding with metal via the azomethine nitrogen and phenolic oxygen atoms. The P-XRD analysis of the complexes revealed a variety of crystal systems including monoclinic and tetragonal structures. Compared to azo-Schiff base ligand, the Cu(II) and Zn(II) complexes showed better neuroprotective activity. The Co(II) complex exhibit higher cytotoxicity but less neuroprotective activity. The metal complexes exhibit higher antimicrobial activity than azo-Schiff base ligand. The synthesized metal complexes show better antifungal activity than antibacterial activity.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## AUTHOR CONTRIBUTIONS

All the authors contributed significantly to the manuscript, participated in reviewing/editing and approved the final draft for publication.


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## CONFLICT OF INTEREST

We all authors declare that there is no conflict of interest.

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