INTRODUCTION

Medicinal inorganic chemistry is one of the most rapidly developing areas of pharmaceutical research. It attracted much attention after the prototypical success story of cisplatin [cis(diaminedichloroplatinum(II))] for treating solid tumors (Sartaj Tabassum et al., 2012). Despite its success, there were serious concerns of this drug regarding its toxicity issues, intrinsic resistance, narrow spectrum of activity for phenotypes of cancer and patient compliance (Kelland et al., 2000). Therefore, studies pertaining to essential metalloelements received much attention in contrast to Pt, since the drugs designed and synthesized from endogenous metal ions may be less harmful and more prone to antiproliferative activity against tumors and many other diseases (Zhang et al., 2003, Ronconi et al., 2007). Among all metals, copper is an attractive prospect, being an essential trace element which is required for normal cellular activity as a cofactor for many enzymes. However, role of copper is much more complex because it can also promote nucleic acid cleavage and therefore has been utilized as metallodrug to cause DNA damage. More recently, copper-based heteronuclear complexes were explored for displaying intriguing nuclease properties (Liu et al., 2004). The Schiff base properties of nickel complex are high in demand in bioinorganic chemistry as well as in the redox enzyme reactions (Claudio Mendicute Fierro et al., 2011). Nickel complexes are used in heterogeneous catalysis, electroplating, and in making pigments and ceramics. A number of nickel complexes of Schiff bases have been seen to possess fungicidal and bacterial activity. Applications of vanadium compounds in medicine have focused their activity, in vitro and in vivo, in the treatment, of insulin deficiency type I diabetes, and insulin tolerance, type 2 diabetes. Besides the insulin-like activity of oxidovanadium (V) and oxido vanadium (IV) compounds (Tudor Rosu et al., 2010), its presence in vanadium dependent haloperoxidases has particularly stimulated the search for structural and functional models. At the onset, the present work stems from our interest to develop the synthesis, characterization, DNA cleavage, antibacterial, antifungal and antioxidant abilities of Cu(II), Ni(II), VO(II) complexes. Complexes were structurally characterized by various physico chemical techniques. All the synthesized compounds show significant biological activity.

Experimental Section

All the chemicals were of reagent grade and the solvents were distilled before use according to the standard procedure. Metal salts, 3, 3′-dihydroxybenzidine, diacetyl and 2-aminothiophenol were obtained from Aldrich and used as received. Ethanol, Acetonitrile, DMSO and DMF were used as solvents purchased from Merck and Loba chemicals.
Physical Measurements

Elemental analysis was carried out on a Carlo Erba Model 1106 elemental analyzer. Molar conductivity was measured by using an ELICO CM 185 conductivity Bridge using freshly prepared solution of the complexes in DMF solution. IR spectra were recorded on a Thermo Nicolet, Avatar 370 model spectrophotometer on KBr disks in the range 4000–400 cm⁻¹. Electronic spectral studies were carried out on a Perkin Elmer Lambda-25 spectrophotometer in the range 200–800 nm. The magnetic studies were carried out at room temperature on a Gouy balance calibrated with Hg[Co(SCN)₄]₂. EPR spectra were recorded on an E-112 ESR spectrometer at X-band microwave frequencies for powdered samples. The ¹H NMR spectra were recorded in DMSO-d₆ on a BRUKER ADVANCED III 400 MHz spectrophotometer using TMS as an internal reference.

Synthesis of 4, 4'-Bis-[2-(mercapto-phenylimino)-1-methyl-propylideneamino]-biphenyl-3, 3'-diol

An ethanolic solution of 3, 3'-dihydroxybenzidine (1 mM) in 10 mL of ethanol, diacetyl (2 mM) and 2-aminothiophenol (2 mM) in 20 mL of ethanol was mixed. The resultant mixture was refluxed for 2 h as shown in Figure 1. The solid product formed was filtered and recrystallized from ethanol.

After partial evaporation of the solvent, solid (70–80%) metal (II) Schiff base complexes (Figure 2) were separated and dried in vacuum. The analysis results are in good consistency with proposed formulas in Table 1.

Anti-microbial activity

The Schiff base 4, 4'-Bis-[2-(mercapto-phenylimino)-1-methyl-propylideneamino]-biphenyl-3, 3'-diol and its complexes were investigated for anti-bacterial and anti-fungal against Staphylococcus aureus and Bacillus subtilis as gram positive bacteria and Escherichia coli and Klebsiella pneumoniae as Gram-negative and the fungi Fusarium oxysporum and Aspergillus fumigatus by using disc-agar diffusion method (Ekampam Arika et al., 2012). The anti-microbial activity was carried out at Progen Lab at Salem, Tamilnadu (India). The antibiotic Streptomycin was used as standard reference in the case bacteria and Clotrimazole was used as standard anti-fungal reference. The tested compounds were dissolved in DMF (which have no inhibition activity), to get concentration of 100 µg/mL. The test was performed on medium potato dextrose agar contains infusion of 200 g potatoes, 6 g dextrose and 15 g agar (Mohammad Akbar Ali et al., 2002). Uniform size filter paper disks (three disks per compound) were impregnated by equal volume from the specific concentration of dissolved tested compounds and carefully placed on incubated agar surface. After incubation for 36 h at 27°C in the case of bacteria and for 48 h at 24°C in the case of fungus, inhibition of the organism which evidenced by clear zone surround each disk was measured and used to calculate mean of inhibition zones (Poomalai Jayaseelan et al., 2011).

Antioxidant Assay

DPPH radical scavenging assay

4,4'-Bis-[2-(mercapto-phenylimino)-1-methyl-propylideneamino]-biphenyl-3, 3'-diol and its complexes were determined mainly by its free radical scavenging ability on the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals described in the literature (Kavitha et al., 2013). The scavenging ability determines the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm. All of these compounds exhibit free radical scavenging ability at the different concentrations (200, 400, 600, 800, 1000 µg/ mL). The radical scavenging activity was calculated using the following formula:

\[
\% \text{ inhibition} = \frac{[(\text{Absorbance of control} – \text{Absorbance of test Sample})/\text{Absorbance control}]}{\times 100}
\]

IC₅₀ values were calculated for compounds, which exhibited the significant activity. IC₅₀ is defined as concentration sufficient to obtain 50% of maximum scavenging activity.

Methodology for DNA Cleavage Study

Agar gel electrophoresis was used to study the DNA cleavage activity of the complexes. pUC18 plasmid was cultured, isolated and used as DNA for the experiment. The gel electrophoresis experiments were performed by incubation of the samples containing 40 µM pUC18 DNA, 50 µM metal complexes and 50 µM H₂O₂ in tris-HCl buffer (pH 7.2) at 37°C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 µg cm⁻³ ethidium bromide (EB) and photographed under ultraviolet light at 360 nm. All the
experiments were performed at room temperature (Akila et al., 2013).

**RESULTS AND DISCUSSION**

The Schiff base ligand and its Cu(II), Ni(II) and VO(II) complexes have been synthesized and characterized by spectral and elemental analytical data. They are found to be air stable. The ligand is soluble in common organic solvents and all the complexes are freely soluble in acetonitrile, DMF and DMSO but slightly soluble in methanol and ethanol and insoluble in water.

The metal(II) complexes were dissolved in DMF and the molar conductivities of 10^2 M of their solution at room temperature were measured. The lower conductance values (11.3–15.5) of the complexes support their non-electrolytic nature of the compounds (krishna murthy potla et al., 2013).

**IR Spectra**

The coordination mode and sites of the ligand to the metal ions were investigated by comparing the infrared spectra of the free ligand with its metal complexes.

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**Table 1** Physical characterization, analytical data of the ligand and binuclear Schiff base complexes.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molecular Formula</th>
<th>Color</th>
<th>Yield (%)</th>
<th>Melting Point (°C)</th>
<th>Calculated (Found) (%)</th>
<th>$\lambda_m$ (ohm cm) \textsuperscript{-1}</th>
<th>Geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>C$<em>{10}$H$</em>{10}$N$<em>{6}$O$</em>{5}$S$_{2}$</td>
<td>Pale Yellow</td>
<td>83</td>
<td>152</td>
<td>67.75</td>
<td>5.29</td>
<td>9.88</td>
</tr>
<tr>
<td>[CuL]</td>
<td>[Cu$_2$(C$_5$H$_4$N$_2$O$_2$)$_3$]</td>
<td>Dark green</td>
<td>80</td>
<td>&gt;200</td>
<td>(67.54) (5.26) (9.85)</td>
<td>15.5</td>
<td>--</td>
</tr>
<tr>
<td>[NiL]</td>
<td>[Ni$_2$(C$_5$H$_4$N$_2$O$_2$)$_3$]</td>
<td>Brownish blue</td>
<td>75</td>
<td>&gt;200</td>
<td>(55.66) (3.75) (8.11)</td>
<td>13.3</td>
<td>--</td>
</tr>
<tr>
<td>[VO-L]</td>
<td>[VO$_2$(C$_5$H$_4$N$_2$O$_2$)$_3$]</td>
<td>Blackish brown</td>
<td>75</td>
<td>&gt;200</td>
<td>(56.46) (3.82) (8.23)</td>
<td>11.3</td>
<td>--</td>
</tr>
</tbody>
</table>

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**Table 2** Infrared and Electronic Spectral Data

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Free-OH (cm$^{-1}$)</th>
<th>(C=N) (cm$^{-1}$)</th>
<th>(C=S) (cm$^{-1}$)</th>
<th>(V=O) (cm$^{-1}$)</th>
<th>(M=N) (cm$^{-1}$)</th>
<th>(M−O) (cm$^{-1}$)</th>
<th>$\lambda_{max}$</th>
<th>Geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>C$<em>{10}$H$</em>{10}$N$<em>{6}$O$</em>{5}$S$_{2}$</td>
<td>3700</td>
<td>1612</td>
<td>744</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>[Cu$_2$(C$_5$H$_4$N$_2$O$_2$)$_3$]</td>
<td>--</td>
<td>1605</td>
<td>732</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Square planar</td>
</tr>
<tr>
<td>[Ni$_2$(C$_5$H$_4$N$_2$O$_2$)$_3$]</td>
<td>1595</td>
<td>756</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>Square planar</td>
</tr>
<tr>
<td>[VO$_2$(C$_5$H$_4$N$_2$O$_2$)$_3$]</td>
<td>1597</td>
<td>745</td>
<td>987</td>
<td>446</td>
<td>576</td>
<td>510, 570, 610</td>
<td>15.5</td>
<td>Square pyramidal</td>
</tr>
</tbody>
</table>

---

**Table 3** Various Bond Lengths of [Cu$_2$(C$_{14}$H$_{30}$N$_{6}$O$_{8}$S$_{2}$)] complex.

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Actual bond length</th>
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</thead>
<tbody>
<tr>
<td>N(8)-Cu(53)</td>
<td>1.846</td>
</tr>
<tr>
<td>N(14)-Cu(54)</td>
<td>1.846</td>
</tr>
<tr>
<td>N(19)-Cu(53)</td>
<td>1.846</td>
</tr>
<tr>
<td>N(20)-Cu(54)</td>
<td>1.846</td>
</tr>
<tr>
<td>O(34)-Cu(53)</td>
<td>1.872</td>
</tr>
<tr>
<td>O(35)-Cu(54)</td>
<td>1.798</td>
</tr>
<tr>
<td>S(36)-Cu(54)</td>
<td>2.201</td>
</tr>
</tbody>
</table>

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**Table 4** Various Bond Angles of [Cu$_2$(C$_{14}$H$_{30}$N$_{6}$O$_{8}$S$_{2}$)] complex

<table>
<thead>
<tr>
<th>Atoms</th>
<th>Actual bond angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(12)-N(8)-Cu(53)</td>
<td>111.00</td>
</tr>
<tr>
<td>C(16)-N(8)-Cu(53)</td>
<td>111.00</td>
</tr>
<tr>
<td>C(11)-N(14)-Cu(54)</td>
<td>111.00</td>
</tr>
<tr>
<td>C(15)-N(14)-Cu(54)</td>
<td>111.00</td>
</tr>
<tr>
<td>C(18)-N(19)-Cu(53)</td>
<td>110.998</td>
</tr>
<tr>
<td>C(25)-N(19)-Cu(53)</td>
<td>111.00</td>
</tr>
<tr>
<td>C(17)-N(20)-Cu(54)</td>
<td>111.00</td>
</tr>
<tr>
<td>C(27)-N(20)-Cu(54)</td>
<td>110.998</td>
</tr>
<tr>
<td>C(24)-S(33)-Cu(53)</td>
<td>95.150</td>
</tr>
<tr>
<td>C(3)-O(34)-Cu(53)</td>
<td>110.213</td>
</tr>
<tr>
<td>C(10)-O(35)-Cu(53)</td>
<td>114.287</td>
</tr>
<tr>
<td>C(28)-S(36)-Cu(54)</td>
<td>90.833</td>
</tr>
</tbody>
</table>
Electronic Spectra and Magnetic Data

The geometry of the metal complexes has been deduced from electronic spectra and magnetic data of the complexes. The electronic spectra of the complexes were recorded in DMF solution. All the complexes show the high energy absorption band in the region 465-485 nm. This transition may be attributed to the charge transfer band. The electronic spectrum of copper(II) complex displays the d-d transition band in the region 589 nm (Raman et al., 2012) which is due to $^1B_{1g} \rightarrow ^2A_{1g}$ transition. This d-d transition band strongly favors a square-planar geometry around the metal ion.

Copper complex show $\mu_{eq}$ value in the range 1.1/1 B.M. which is close to the spin only value of 1.73 B.M. The lower value of magnetic moment at room temperature is consistent with square planar geometry around the metal ions. The absorption spectrum of nickel(II) complex displays two d-d bands at 544 and 622 nm. These bands correspond to $^1A_{1g} \rightarrow ^1A_{2g}$, $^1A_{1g} \rightarrow ^1B_{1g}$ transitions respectively, being characteristic of square-planar geometry (Claudio Mendicute Fierro et al., 2011), also Ni (II) complex is diamagnetic in nature. The electronic spectrum of VO (II) complex displays three d-d transition bands in the region 510, 570, and 610 nm (Table 2) which are assigned to $d_x^2-y^2-d_z^2$, $d_y^2-d_z^2$ and $d_x^2-d_y^2$ transitions respectively. This indicates that the complex of VO (II) is five coordinate and probably an square-pyramidal geometry (Leeavathly et al., 2009), which is also supported by its magnetic susceptibility value (1.69 BM).

$^1$H NMR Spectral Studies

The $^1$H NMR spectrum of the 4, 4′-Bis-[2-(2-mercapto-phenylimino)-1-methyl-propylideneamino]-biphenyl-3, 3′-diol was recorded in DMSO-d$_6$ at room temperature. The signal at $\delta$ (12.23) (s, 1H) is assigned to thiophenolic proton of (–SH–) group and the signal at $\delta$ (8.76) (s, 1H) is assigned to azomethine proton, respectively. The aromatic protons at $\delta$(6.16–8.54) shifted downfield in the complexes (Mustafa Dolaz et al., 2009) respectively.

Electron paramagnetic resonance spectra

The EPR spectrum of copper complex provides information, important in studying the metal ion environment. The EPR spectra were recorded in DMSO at RT (room temperature). The copper complex exhibited the $g||$ value of 2.264 and $g\perp$ value of 2.066. These values indicate that the Cu(II) lies predominantly in the dx$^2$–y$^2$ orbital, as was evident from the value of the exchange interaction term G, estimated from the expression:

$$G = (g|| - 2)/(g\perp - 2) = 4.0$$

if $G > 4$, the exchange interaction between copper(II) centers in the solid state is negligible. Whereas $G < 4$, a considerable exchange interaction occurs in the solid state complexes. The observed value for the exchange interaction parameter for the copper complex ($G \approx 4.1$) suggests that the Cu (II) complex is in square planar geometry and the unpaired electron is present in the dx$^2$–y$^2$ orbital. This result also indicates that the exchange coupling effects are not operative in the present complex (Sulekh Chandra, et al., 2005).

Figure 3 3D Structure of Compound of [Cu$_{2}$][C$_{8}$H$_{8}$N$_{4}$O$_{5}$]complex.

Figure 4 The in vitro antibacterial activity of Schiff base and its metal complexes. Standard = Streptomycin, Inhibition Zone in mm, Concentration 100 µg/mL.

Figure 5 The in vitro antibacterial activity of Schiff base and its metal complexes. Standard = Clotrimazole, Inhibition Zone in mm, Concentration 100 µg/mL.

Figure 6 DPPH Scavenging Activity of ligand and Its Complexes
**Molecular Modelling**

The molecular modelling of [Cu₂(C₃H₆N,O₃S₂)] complex as a representative, is based on its square planar structure with tetradentate ligand. This Molecular modeling structure also shows the stereochemistry of the complex. The details of the bond lengths (Å) and bond angles (°) as per the 3D structure (Figure 3) are given in Tables 3 and 4 respectively (Maurya et al., 2008). From the observed bond lengths and bond angles thus the proposed structure is square planar geometry and is suitable.

**Antibacterial assay**

The Schiff base complexes have provoked wide interest because they possess a diverse spectrum of biological and pharmaceutical activities. The synthesized ligand and its complexes were tested for their in vitro antibacterial activity. *Streptomycine* was used as standard here. They were tested against the bacteria (Gram-positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis* and two Gram-negative bacteria like *Escherichia coli* and *Klebsilla pneumonia*) by disc diffusion method. The antibacterial activity of the newly synthesized compounds as shown in the Figure 4. The results indicate that the ligand exhibits moderate antibacterial activity with respect to the complexes against the same microorganisms under identical experimental conditions. Further, the antibacterial action of Schiff base ligand may be significantly enhanced on the presence of azomethine groups which have chelating properties. These properties may be used in metal transport across the bacterial membranes or to attach to the bacterial cells at a specific site from which it can interfere with their growth. The copper complex shows better antibacterial activity against the tested microorganisms than the other complexes. It may be attributed to the atomic radius and the electronegativity of Cu(II) ions. Current studies reveal that the high atomic radius and electronegative metal ions in their metal complexes exhibit high antimicrobial activity. Higher electronegativity and large atomic radius decreases the effective positive charges on the metal complex molecules which facilitates their interaction with the highly sensitive cellular membranes towards the charged particle (krishna murthy potla et al., 2013).

**Antifungal activity**

The Schiff base and its metal complexes were screened for their antifungal activity against *Fusarium oxysporum* and *Aspergillus fumigates*. *Clotrimazole* was used as standard here. The results indicate that the metal complexes exhibit higher antifungal activity than the ligand as shown in the Figure 5. Such increased activity on metal chelation can be explained on the basis of Tweedy’s chelation theory. Chelation reduces the polarity of the metal ion considerably because of the partial sharing of its positive charge with the donor groups and also due to π -electron delocalization on the whole chelating ring. The lipids and polysaccharides are some important constituents of the cell wall and membranes which are preferred for metal ion interaction. Apart from this, the cell wall also contains many phosphates, carbonyl and cystenyl ligands which maintain the integrity of the membrane by acting as a diffusion barrier and also provide suitable sites for binding. Furthermore, increased lipophilicity enhances the penetration of the complexes into lipid membrane and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of the proteins which restricts further growth of the organism (Raman et al., 2011).

**Antioxidant activity**

**DPPH Free radical scavenging activity**

The variation of DPPH radical scavenging activity with concentration of test compounds is represented in Figure 6 and 7. In this study, the ligand and its Cu (II), Ni(II) and VO(II) complexes were screened for their DPPH radical scavenging activity. The copper (II) complex shows very good activity when compared to Ni (II) and VO (II) complexes and its ligand, respectively. The IC₅₀ values were determined for all compounds and reported in Figure 6 and 7. From the results, it was found that the IC₅₀ value of [Cu₂(C₃H₆N,O₃S₂)] is 5.89 mg/ml show significant activity compared to remaining complexes and ligand. Ascorbic acid is used as standard. The order of the scavenging activity of all the complexes according to their IC₅₀ values is given below:

Ascorbic acid > [Cu₂(C₃H₆N,O₃S₂)] > [Ni₂(C₃H₈N₂O₂S₂)] > [VO₂(C₃H₈N₂O₂S₂)] > C₃H₄NiO₂S₂

**Chemical nuclease activity**

The study on the cleavage capacity of transition metal complex to DNA is considerably interesting as it can contribute to understanding the toxicity mechanism of them and to develop novel artificial nuclease. DNA cleavage is controlled by relaxation of super coiled circular form of pUC18 DNA into nicked circular form and linear form. When circular plasmid DNA is conducted by electrophoresis the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slowed moving open circular form (Form II). A series of complexes were screened for DNA cleavage activity and the IC₅₀ values are given in Tables 3 and 4 respectively (Maurya et al., 2008).
II). If both strands are cleaved a linear form (Form III) will be generated that migrates in between (Ekamparam Akila et al., 2013). Figure 8 shows the result of gel electrophoretic separations of plasmid pUC18 DNA induced by an addition of metal (II) complexes in the presence of H$_2$O$_2$. All super coiled (Form I) DNA was cleaved to form the mixture of Form II and Form III with the addition of the complex. These phenomena imply that Cu(II), Ni(II) and VO(II) complexes induce intensively the cleavage of plasmid pUC18 DNA in the presence of H$_2$O$_2$. These observations suggested that all the complexes effectively cleave DNA.

**CONCLUSION**

In this paper, the coordination chemistry of a Schiff base ligand obtained from the reaction of 3, 3'-dihydroxybenzidine, diacetyl and 2-aminothiophenol is described. Cu(II), Ni(II) and VO(II) complexes have been characterized by spectral and analytical data. The IR, electronic transition data lead to the conclusion that the Cu(II) and Ni(II) ion assumes a square planar geometry and the VO(II) are square pyramidal in nature. In all the complexes, the ligand acts as tetradentate. The complexes are capable of cleaving pUC18 DNA in the presence of H$_2$O$_2$. The antimicrobial and antioxidant screening data reveal that the complexes have higher activity than the free ligand.

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**References**


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