SYNTHESIS, SPECTROSCOPIC AND ANTIMICROBIAL STUDIES OF BINUCLEAR SCHIFF BASE COMPLEXES WITH N$_2$O$_2$ DONOR GROUPS

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ABSTRACT

A binuclear Schiff base, H$_2$L, ligand synthesized by the reaction of 5-bromo-3-fluorosalicylaldehyde with 4, 4’-diaminodiphenylmethane in the molar ratio 2:1 forms complexes with transition metal ions such as Cu(II), Ni(II), V(IV) and Co(II). The elemental analysis, conductivity, IR, electronic, $^1$H NMR, LC-MS and magnetic susceptibility measurements were used to characterize the isolated ligand and its metal complexes. The ligand acts as dibasic with N$_2$O$_2$ tetradentate sites and two ligands coordinate with two metal ions to form binuclear complexes. The $^1$H - NMR data reveal that the Schiff base exists in enol – iminic form. The binding sites are the nitrogen atoms of azomethine groups and oxygen atoms of the phenolic groups. Substitution in phenyl ring of the complexes produces shift in the azomethine $\nu$(C=N) stretching vibrational frequency. The non-electrolytic behavior of complexes indicates the absence of counter ions. EPR spectra provided further information to confirm the binuclear structure. On the basis of electronic spectral data and magnetic susceptibility measurements, suitable geometry has been proposed for each complex. The Schiff base ligand and its metal complexes were tested against four pathogenic bacteria (S. aureus and B. subtilis) as Gram – positive bacteria (E. coli and K. pneumoniae) as Gram – negative bacteria and one pathogenic fungi (A. fumigatus) to assess their antimicrobial properties.

KEYWORDS: Schiff base, binuclear, anti-microbial activity.
INTRODUCTION
Schiff bases have played an important role in the development of coordination chemistry as they readily form stable complexes with most of the transition metals. In the area of bioinorganic chemistry, interest in Schiff base complexes has centered on the role of such complexes in providing synthetic models for the metal containing sites in metallo-proteins and enzymes \(^1\). Metal complexes of Schiff bases have been designed and synthesized to explore their pharmacological activity and more recently antitumour activity \(^2\). Salicylaldimine Schiff bases have emerged as an important class of ligands not only due to their novel structural features but also in view of their immense biological properties \(^3\). The binucleating Schiff base complexes have numerous applications such as in the treatment of cancer \(^4\), as antibactericide agents \(^5\), as antifungicide agents \(^6\) and for other biological properties \(^7\). Several applications have been related for these complexes in chemical analysis, homogeneous and heterogeneous catalysis for oxidation and polymerization of organic compounds \(^8\). The interest in Schiff base complexes as analytical reagents is increasing since they enable simple and inexpensive determinations of different organic and inorganic substances. The term “binucleating ligands” was first introduced in 1970 by Robson to describe the class of polydentate chelating ligands which able to bind simultaneously two metal ions. Among many different types of binucleating agents, the salen ligands attracted particularly wide attention of scientists. The high affinity for the chelation of Schiff bases towards the transition metal ions is utilized in preparing their solid complexes. In view of recent interest in the biological activities of the metal – ligand chelates involving Schiff base ligands, we started to study the biological activities of Schiff base complexes derived from tetridentate ligands involving $\text{N}_2\text{O}_2$ donor atoms.

This paper describes the synthesis, spectral and magnetic studies of Schiff base derived from 5-bromo-3-fluorosalicylaldehyde and 4, 4’– dianinodiphenylmethane, its binuclear Cu(II), Ni(II), V(IV) and Co(II) complexes. Indeed, the biological activity of the ligand and its complexes were screened against selected kinds of bacteria and fungi.

MATERIALS AND METHODS
Experimental
Chemicals were purchased from Sigma-Alrich and were used without further purification. Methanol, Ethanol, Dimethylsulphoxide and DMF were HPLC grade and were used after purification by the standard methods as described in the literature \(^9\).
Physical measurements
C, H, N micro analysis was carried out with Elementar Vario EL(III). The FT-IR spectra (4000-400 cm\(^{-1}\)) of the compounds were recorded with Bruker Optik GmbH-Tensor 27 of OPUS version 6.5 with solid KBr disc. The electronic spectra were recorded at 300 K on a Perkin-Elmer Lambda 40 (UV-Vis) spectrometer DMF as solvent in the range 200-800 nm. \(^1\)H NMR spectra was recorded on a Bruker 300 MHz spectrometer using tetramethylsilane as an internal standard. Mass spectra of the ligand were recorded on an Agilent6330 Ion Trap mass spectrometer. The molar conductance values at room temperature were measured in DMF (10\(^{-3}\) mol dm\(^{-3}\)) on an Elico digital direct conductivity meter model CM-180. The magnetic studies were carried out at room temperature on a PAR vibrating sample magnetometer (Model-155). EPR spectra were recorded on a Bruker EMX plus, X-band spectrometer (9.86 GHz) with 100 KHz modulation frequency. The purity of the ligand and its complexes were checked by TLC.

Synthesis of the Schiff base, H\(_2\)L, ligand
The ligand was synthesized by adding 5-bromo-3-fluorosalicylaldehyde (2 mmol) dissolved in methanol (25 ml) to 4, 4′– dianinodiphenylmethane (1 mmol) in methanol (25 ml). The reaction mixture was heated to reflux for 3 hr. The orange product was filtered off, washed with methanol then ether and air dried. The ligand was kept in desiccator until used. The yield was 80 %.

The synthetic procedure of Schiff base ligand, H\(_2\)L is illustrated in “Fig.1”.

Synthesis of the Schiff base metal complexes
A methanolic solution of the metal salt (25 ml) was added gradually to a hot methanolic solution of the deprotonated ligand (25 ml) in the molar ratio 1:1. Triethylamine was used for the deprotonation of the ligand. The reaction mixtures of the deprotonated H\(_2\)L, ligand with metal salts were heated to reflux for 3 hr. The resulting precipitates were filtered off, washed with hot methanol then diethyl ether and dried over anhydrous CaCl\(_2\).
Fig. 1 Synthesis of Ligand and its complexes

**Antimicrobial Activities**

The standardized disc-agar diffusion method\(^{[10]}\) was followed to determine the activity of the synthesized compounds against the sensitive organisms *S. aureus* and *B. subtilis* as Gram – positive bacteria *E. coli* and *K. pneumoniae* as Gram – negative and the fungi *A. fumigatus*. The antibiotic ampicillin was used as standard reference in the case of bacteria and fluconazole was used as a standard antifungal reference. The tested compounds were dissolved in DMSO (which has no inhibition activity) to get concentration of 2 and 1 mg mL\(^{-1}\). The test was performed on medium potato agar (PDA) which contains infusion of 200 g
potatoes, 6 g dextrose and 15 g agar. Uniform size filter paper disks (three disks per compound) were impregnated by equal volume (10 μL) from the specific concentration of dissolved test compounds and carefully placed on incubated agar surface. After incubation for 36 h at 27 °C in the case of bacteria and 48 h at 24 °C in the case of fungi, inhibition of the organisms which evidenced by clear zone surround each disk was measured and used to calculate mean of inhibition zones.

RESULTS AND DISCUSSION
The resulting solids are intensely coloured and stable in air. The ligand is partially soluble in common organic solvents and the complexes are soluble only in DMF and DMSO.

Elemental analysis
The elemental analysis is in good agreement with that calculated for the proposed formula (Table 1).

Table: 1. Physical and analytical data for the Schiff base, H2L, ligand and its metal complexes

<table>
<thead>
<tr>
<th>S. No</th>
<th>Compound</th>
<th>Molecular weight</th>
<th>Colour</th>
<th>Yield (%)</th>
<th>M.P (°C)a</th>
<th>Elemental analysis, Found (Calc.)b [%, %]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>C27H18F2Br2N2O2</td>
<td>599.8</td>
<td>Orange</td>
<td>80</td>
<td>240</td>
<td>54.12 (54.02)</td>
</tr>
<tr>
<td>2</td>
<td>[Cu2(C27H16F2Br2N2O2)2]</td>
<td>1322.7</td>
<td>Dark brown</td>
<td>60</td>
<td>&gt;250</td>
<td>48.90 (48.99)</td>
</tr>
<tr>
<td>3</td>
<td>[Ni2(C27H16F2Br2N2O2)2]</td>
<td>1312.96</td>
<td>Light green</td>
<td>62</td>
<td>&gt;250</td>
<td>49.36 (49.35)</td>
</tr>
<tr>
<td>4</td>
<td>[V2O2(C27H16F2Br2N2O2)2]</td>
<td>1329.48</td>
<td>Green</td>
<td>65</td>
<td>&gt;250</td>
<td>48.76 (48.74)</td>
</tr>
<tr>
<td>5</td>
<td>[Co2(C27H16F2Br2N2O2)2]</td>
<td>1313.46</td>
<td>Greenish brown</td>
<td>65</td>
<td>&gt;250</td>
<td>49.36 (49.34)</td>
</tr>
</tbody>
</table>

aMelting point of the compound at their decomposition
bElemental analysis for C, H and N were within ± 0.4% of the theoretical value.

Molar conductance
The conductivity measurements were carried out in 10⁻³ mol/dm³ DMF solution at 25 °C. The room temperature molar conductivity values are given in table 3. These low values indicate that all the complexes are non-electrolytes due to the absence of any counter ions in their structure [11].
$^1$H NMR Spectra
The $^1$H NMR Spectra of the free ligand was recorded in DMSO-d$_6$. The free ligand exhibits sharp low field signal for the phenolic proton at 13.46 ppm “Fig.2”. This shows that the Schiff base forms intramolecular hydrogen bonds $^{[12]}$ involving the phenolic proton and the azomethine nitrogen atom, thus implying a phenol-iminic structure. Signal for the methine proton of the azomethine group –N=C(H)- was observed between 8.5-8.9 ppm. The peaks in the region of 7.5-6.9 ppm were assigned to chemical shifts for protons of the aromatic ring. The total number of protons present in the Schiff base exhibited signals of the protons in their expected regions and these data are in good agreement with that previously reported for similar compounds.

![Fig.2 $^1$H NMR spectra of Ligand](image)

Mass Spectra
The mass spectrum of the Schiff base, H$_2$L ligand, revealed the molecular ion peak at m/e = 599.2 which is coincident with the formula weight 599.8 and support the identity of the structure. “Fig.3” depicts the mass spectrum of the H$_2$L ligand.
IR spectra

IR spectra of the Schiff base ligand was compared with that of the complexes in order to determine the coordination sites that may involve in chelation. The O-H stretching frequency of the ligand exhibits broad weak intensity band in the 2500-3500 cm\(^{-1}\) range, which is assigned to the intra molecular hydrogen bond O-H…N=C. This band disappeared in the spectra of the complexes. A strong sharp absorption band around 1620 cm\(^{-1}\) in the spectrum of the ligand can be assigned to the C=N stretching. In all the complexes, this band is shifted to lower frequencies in the range 1620-1600 cm\(^{-1}\) upon complexation with the metal, which can be attributed to the coordination of the imine nitrogen to the metal centre \(^{13}\). The frequency of the aromatic carbon and phenolic oxygen of the free ligand at 1282 cm\(^{-1}\) undergoes shifts (\(\Delta \nu = 10-30 \text{ cm}^{-1}\)) in the binuclear complexes, indicating that the Schiff base is bonded to the metallic ions through the phenolic oxygen atoms. The band at 969 cm\(^{-1}\) is assigned to \(\nu (V=O)\), this band is observed as a new peak for the complexes and is not present in the spectrum of free ligand. Finally, the appearance of two non-ligand bands in the two ranges 500-560 cm-1 and 440-470 cm\(^{-1}\) in all the complexes could be assigned to the stretching frequencies of \(\nu (M–O)\) and \(\nu (M–N)\) respectively \(^{14}\). Therefore from the IR spectra, it is concluded that H\(_2\)L behaves as a tetradentate ligand with ONNO donor sites coordinating to the metal ions via azomethine N and deprotonated phenolic O atoms (Table 2).

Fig.3 Mass spectra of Ligand
Table. 2. Infrared spectral data of the ligand and its metal complexes (ν in cm\(^{-1}\)) and their assignments

<table>
<thead>
<tr>
<th>Compound</th>
<th>ν(C=N)</th>
<th>ν(C−O)</th>
<th>ν(O−H)</th>
<th>ν(M−O)</th>
<th>ν(M−N)</th>
<th>ν(VO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(_2)L</td>
<td>1620</td>
<td>1282</td>
<td>2919</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>1</td>
<td>1605</td>
<td>1330</td>
<td>***</td>
<td>555</td>
<td>452</td>
<td>***</td>
</tr>
<tr>
<td>2</td>
<td>1604</td>
<td>1325</td>
<td>***</td>
<td>550</td>
<td>450</td>
<td>***</td>
</tr>
<tr>
<td>3</td>
<td>1601</td>
<td>1322</td>
<td>***</td>
<td>553</td>
<td>455</td>
<td>969</td>
</tr>
<tr>
<td>4</td>
<td>1603</td>
<td>1319</td>
<td>***</td>
<td>558</td>
<td>447</td>
<td>***</td>
</tr>
</tbody>
</table>

Electronic, EPR spectra and magnetic moment measurements

UV-Vis spectra provide the most detailed information about the electronic structure. The UV-Vis spectrum of the Schiff base ligand (H\(_2\)L) exhibits two charge transfer (CT) bands at 275 nm and 330 nm attributed to π−π* and n−π* transition within the Schiff base ligand. In the spectrum of the complexes, the CT band at 260 nm remains as such, in agreement with the π−π* transition of the Schiff base ligand. The band observed at 325 nm observed in the spectrum of free ligand (H\(_2\)L) is red shifted to 380 – 395 nm in complexes in the form of ligand to metal charge transfer (LMCT) transition (Table 3). Similarly much weaker and less well defined broad bands are found in the spectrum of the complexes at 500-650 nm expected which are assigned to the d-d transitions. The absorption spectrum of copper complexes show a broad band at about 575 nm attributed to \(^2\)B\(_{1g}\) → \(^2\)A\(_{2g}\) transition which is well within the range of 610-555 nm expected for square planar CuO\(_2\)N\(_2\) chromophore. The magnetic moment (μ\(_{\text{eff}}\)) for this complex was found to be 1.78 B.M. per copper ion which was in usual range for square planar copper complexes and indicating no direct interaction between two copper centers in the solid state at room temperature\(^{[15]}\).

The X-band EPR spectra of complex 1 was recorded in the solid state at 25 °C. The spectrum (Fig. 4) of the Cu complex exhibits g∥ at 2.41 and g⊥ at 2.08. The shape of the spectrum is consistent with the square-planar geometry around each Cu(II) centre in the complex\(^{[16]}\).
The parameter $G$, determined as $G = \frac{(g||-2)}{(g\perp-2)}$, which measures the exchange interaction between the metal center has been calculated. According to Hathaway et al. [17] if $G > 4$, the exchange interaction is negligible, but $G < 4$ indicates considerable exchange interaction in the solid complexes. The synthesized Cu(II) complex reported in this paper give the “G” values which are greater than 4 indicating the exchange interaction is absent in solid complex “Fig. 4”.

The electronic spectra of binuclear Ni(II) complexes show a band at 505 nm that may be assigned to $^2A_{1g} \rightarrow ^2A_{2g}$ transition, indicating a square planar environment around nickel(II) metal ions [18] which is confirmed by the diamagnetic properties of the complex.

The binuclear vanadyl complex exhibits only one band in addition to the intense charge transfer band, in the 595 nm region owing to the $^2B_{2g} \rightarrow ^2E$ transition characteristic of square pyramidal geometry. The magnetic moment data of V(IV) complex was 1.79 B.M. which confirms the binuclear structure of the complex [19].

The cobalt(II) complex shows two weak bands from d-d transitions were observed at 615 and 655 nm which may be assigned to $^4A_2 (F) \rightarrow ^4T_1 (F)$ correspond to a tetrahedral environment around the Co$^{2+}$ ion. The magnetic moment of Co(II) complex was found to be 4.31 B.M. which is at the lower end of magnetic moments expected for tetrahedral Co(II) complex suggest the dimeric nature of the complex [20,21].
Table 3. Electronic spectral data and magnetic moment values of the metal complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Molar conductance (ohm⁻¹ cm² mol⁻¹) in DMF</th>
<th>Magnetic moments μ_eff (B.M.) (^a)</th>
<th>Transitions</th>
<th>Tentative assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>---</td>
<td>260</td>
<td>330</td>
<td>---</td>
</tr>
<tr>
<td>1</td>
<td>12.2</td>
<td>1.78</td>
<td>275</td>
<td>390</td>
</tr>
<tr>
<td>2</td>
<td>8.5</td>
<td>Diamagnetic</td>
<td>280</td>
<td>385</td>
</tr>
<tr>
<td>3</td>
<td>10.5</td>
<td>1.79</td>
<td>278</td>
<td>380</td>
</tr>
<tr>
<td>4</td>
<td>9.0</td>
<td>4.31</td>
<td>285</td>
<td>395</td>
</tr>
</tbody>
</table>

Antimicrobial Activity

To contribute in the field of bioinorganic chemistry, the Schiff base ligand, H\(_2\)L and its metal complexes were evaluated for antimicrobial activity against two strains Gram-positive bacteria (S. aureus and B. subtilis), Gram-negative bacteria (E. coli and K. pneumoniae) and fungi (A. fumigatus). The obtained antimicrobials are presented in Table 4. The Schiff base ligand was found to be biologically active. Table 4 shows that all metal complexes exhibit antimicrobial activity in one or more strain and enhanced it comparing with the parent Schiff base. Complexes 1, 2 and 4 showed high activity than complex 3. These complexes showed a high activity in Gram-positive, Gram – negative bacteria and intermediate activity for fungi in high and low concentrations. The activity of the Schiff base ligand and its metal complexes increases as the concentration increases because it is a well known fact that concentration plays a vital role in increasing the degree of inhibition (Table 4). The increase in antibacterial activity of the metal chelation may be due to the effect of the metal ion on the normal cell process. A possible mode for toxicity increase may be considered in the light of the Tweedy’s chelation theory [22].

According to this theory chelation considerably reduces the polarity of the metal ion because its positive charge is partially shared with the donor groups, and π electron delocalization over the entire chelate ring takes place. Such chelation could enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layers of cell membranes. Generally, it is suggested that the chelated complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms.
The structure of the reported compounds also seems to be the principal factor influencing the antimicrobial activity. Other factors such as solubility, conductivity and dipole moment which are affected by the presence of metal ion, may also be possible reasons for increasing the biological activity of the metal complexes as compared to the ligand from which they are derived.

Table. 4. Antimicrobial activity of the Schiff base, H₃L, ligand and its Cu(II), Ni(II), V(IV), and Co(II) complexes against different bacteria and fungi.

<table>
<thead>
<tr>
<th>Ligand / Complexes</th>
<th>Diameter of growth of Inhibition Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram-positive bacteria</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td>H₃L</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>30</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>35</td>
</tr>
</tbody>
</table>

CONCLUSION

In the present study, the Schiff base, H₃L, ligand is dibasic N₂O₂ tetradentate sites. The Schiff base was allowed to react with metal ions in 1:1 molar ratio (ligand:metal ion) to form the binuclear complexes. For this ligand, copper(II) and nickel(II) exhibit a square planar geometry, oxovanadium(IV) exhibits square pyramidal and tetrahedral geometry for cobalt(II) complex respectively. The binding sites are the azomethine nitrogen atoms and the phenolic oxygen atoms. The ligand and its metal complexes shows enhanced moderate antimicrobial activity compared with standard antifungal and antibacterial agents.

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REFERENCES


21. Gokhan Ceyhan, Cumali C, Elik, Serhan Urus, Ibrahim Demirtas, Mahfuz Elmastas, Mehmet Tumer, Antioxidant, electrochemical, thermal, antimicrobial and