

# Synthesis, Characterization and Antimicrobial Activity of Copper(II) Complexes of some Ortho-substituted Aniline Schiff Bases; Crystal Structure of Bis(2-methoxy-6-imino)methylphenol Copper(II) Complex

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## ABSTRACT

This study presents the synthesis, characterization and antimicrobial activity of copper(II) complexes of some ortho-substituted aniline Schiff bases (L<sup>1</sup>–L<sup>8</sup>). The Schiff bases and their respective copper(II) complexes were characterized by a combination of elemental analysis, infrared and UV/Visible studies. The structures of the ligands were also confirmed from <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data. The infrared and electronic transition studies showed that the ligands are bidentate coordinating *via* the imine nitrogen and the phenolic oxygen atoms in a planar configuration. Introduction of aqueous ammonia to the ethanolic solution of L<sup>3</sup>/L<sup>4</sup> and Cu(OAc)<sub>2</sub>·H<sub>2</sub>O aliquot yielded an ammonia-based complex due to the hydrolysis of the imine bond. The crystal structure of the resulting complex indicated a square planar geometry. The copper(II) ion crystallizes in the monoclinic system with a space group of P21/c having a = 10.9309, b = 4.85600, c = 17.7030, α = γ = 90 ° and β = 119.658 °. The geometry is slightly distorted from planarity with O1-Cu1-N1 bond angles of 92.19 ° and 87.8 °. The Schiff base ligands and their respective copper(II) complexes were screened for their *in vitro* antibacterial and antifungal activity against *Escherichia coli* ATCC<sup>®</sup> 8739<sup>™</sup>\*, *Staphylococcus aureus* subsp. *aureus* ATCC<sup>®</sup> 6538<sup>™</sup>\*, *Bacillus subtilis* subsp. *spizizenii* ATCC<sup>®</sup> 6633<sup>™</sup>\* and *Candida albicans* ATCC<sup>®</sup> 2091<sup>™</sup>\*. The *o*-vanillin-based ligands exhibited higher activity than the salicylaldehyde derivatives which were virtually non-active against the tested organisms.

## KEYWORDS

*o*-Vanillin, substituted-aniline, Schiff bases, Cu(II) complexes, antimicrobial activity.

## 1. Introduction

The chemistry of Schiff base compounds has been studied extensively. They have various applications as coordinating ligands,<sup>1–6</sup> as catalysts,<sup>7–9</sup> in electrochemistry,<sup>10–11</sup> and medicinal values. Schiff bases possess antimicrobial<sup>12–19</sup> and anti-inflammatory activity.<sup>20</sup> It has been discovered that the biological activity of some Schiff base ligands became enhanced upon chelation with metal ions.<sup>21–22</sup> In some cases, however, the free ligands do possess higher activity than the corresponding metal complexes,<sup>17</sup> an indication that activity does not depend solely on the presence of metal ions but rather a synergistic effect of many factors. Many Schiff base metal complexes have been prepared and screened for their antimicrobial activity.

Ortho-hydroxysalicylaldimines possess N<sub>2</sub>O<sub>2</sub> donor atoms and consequently, form stable chelates with metal ions.<sup>15,21,23–24</sup> The configuration of the chelate group in the four coordinate complexes may be square-planar, tetrahedral, distorted tetrahedral or distorted trigonal pyramidal with the metal atom at the apex. These configurations depend primarily on the nature of the metal atom and also on the magnitude and symmetry of the ligand field.<sup>25</sup> Many bis-(salicylalimine)Cu(II) complexes Cu(Sal.R)<sub>2</sub>, have been prepared and most of them are considered to be square-planar.<sup>25,26</sup> For (Sal.R)<sub>2</sub>, R being alkyl or aryl groups;

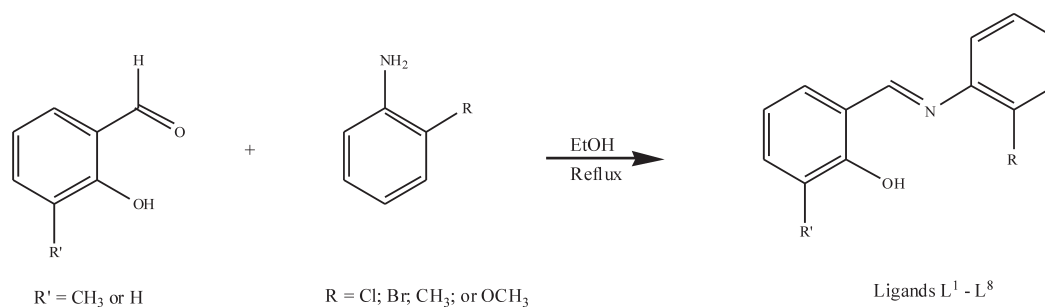
the square-planar configuration is stable, but when there is considerable steric hindrance, the distortion from the planarity may occur.

This study presents the synthesis, characterization and antimicrobial activity of copper(II) complexes of some Schiff base ligands (L<sup>1</sup>–L<sup>8</sup>) derived from 2-hydroxy-3-methoxybenzaldehyde (*o*-vanillin)/2-hydroxybenzaldehyde (salicylaldehyde) and ortho-substituted anilines (R = Cl, Br, CH<sub>3</sub> and OCH<sub>3</sub>). In addition, the crystal structure of bis-(2-methoxy-6-imino)methylphenol copper(II) complex, obtained from the hydrolysis of the methyl and methoxyl derivatives of *o*-vanillin Schiff bases in the presence of aqueous ammonia, is also discussed.

## 2. Materials and Methods

All the chemicals and reagents were of analytical grade and used without further purification. The <sup>1</sup>H- and <sup>13</sup>C-NMR spectra were recorded in CDCl<sub>3</sub> with SiMe<sub>4</sub> as internal standard on a Bruker Avance NMR operating at 400 MHz. The mid-infrared absorption frequencies (4000–700 cm<sup>-1</sup>) were recorded on a PerkinElmer Spectrum 100 FT-IR equipped with universal attenuated total reflectance (ATR) accessory while the far-infrared (700–30 cm<sup>-1</sup>) spectra were recorded in nujol mull on a PerkinElmer Spectrum 400 FT-IR. The UV/Visible spectra were obtained from PerkinElmer Lambda 25 spectrophotometer. The

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Scheme 1 Synthesis of the Schiff base ligands.

elemental analysis, CHN, was done on Vario MICRO V1.6.2 elemental analysen systeme GmbH while the percentage metal content was determined on a PerkinElmer A Analyst atomic absorption spectrometer. Molar conductivity measurement for the complexes was done in DMF, using Az<sup>®</sup> 86555 pH/mV/Cond./TDS/Temp. The melting points (uncorrected) of the compounds were determined using the Galenkemp melting point apparatus. The microorganisms were purchased from Microbiologics (Cape Town, South Africa).

## 2.1. Synthesis of the Schiff Base Ligands

The Schiff base ligands were synthesized according to the general procedure in the literature<sup>27</sup> by condensing *o*-vanillin and salicylaldehyde with 2-chloroaniline, 2-bromoaniline, 2-methylaniline and 2-methoxyaniline, respectively, as presented in Scheme 1. The synthesized ligands are designated as ovan-2-cla, ovan-2-bra, ovan-2-tol, ovan-2-nis, sal-2-cla, sal-2-bra, sal-2-tol and sal-2-nis, corresponding to ligands L<sup>1</sup>–L<sup>8</sup>.

### 2.1.1. Ligand L<sup>1</sup> (ovan-2-cla)

1.522 g (10.00 mmol) of *o*-vanillin was refluxed with 0.91 mL (10.00 mmol) of *o*-chloroaniline in ethanol for 2 h to obtain an orange solution. The solution was concentrated under reduced pressure, and an orange precipitate was obtained. The precipitate was filtered under suction, washed with ethanol and recrystallized from ethanol. It was dried over silica gel in a desiccator. Yield: 1.387 g (53 %);  $\delta\text{H}$  (400 MHz, CDCl<sub>3</sub>): 13.65(1H, s, -OH); 8.68(1H, s, HC=N); 7.52(1H, d, J 7.6 Hz); 7.36(2H, m); 7.25(1H, t, J 8.4 Hz); 7.06(2H, t, J 8.7 Hz); 6.93(1H, t, J 8.0 Hz) and 3.98(3H, s, -OCH<sub>3</sub>).  $\delta\text{C}$  (400 MHz, CDCl<sub>3</sub>): 163.51(HC=N); 152.09(Ar-OCH<sub>3</sub>); 149.06(Ar-OH); 145.49(Ar-N=C); 130.68, 128.23, 128.11, 124.32, 119.04, 115.57(Ar-C) and 56.62(-OCH<sub>3</sub>); (Found: C, 63.82; H, 4.55; N, 5.10 %. Cal. for C<sub>14</sub>H<sub>12</sub>NO<sub>2</sub>Cl: C, 64.27; H, 4.62; N, 5.35 %).

### 2.1.2. Ligand L<sup>2</sup> (ovan-2-bra)

The procedure was the same as for ligand L<sup>1</sup> using *o*-vanillin and *o*-bromoaniline. An orange precipitate was obtained. Yield: 0.890 g (29 %);  $\delta\text{H}$  (400 MHz, CDCl<sub>3</sub>): 13.73(1H, s, -OH); 8.68(1H, s, HC=N); 7.52(1H, d, J 7.5 Hz); 7.34(2H, m); 7.25(1H, t, J 8.4 Hz); 7.06(2H, t, J 8.5 Hz); 6.93(1H, t, J 7.8 Hz) and 3.98(3H, s, -OCH<sub>3</sub>).  $\delta\text{C}$  (400 MHz, CDCl<sub>3</sub>): 163.24(HC=N); 152.02 (Ar-OCH<sub>3</sub>); 148.81(Ar-N=C); 145.27(Ar-OH); 130.68(Ar-Br); 128.27, 128.14, 124.30, 119.00, 115.44(Ar-C) and 56.51(-OCH<sub>3</sub>); (Found: C, 54.84; H, 3.94; N, 4.47 %; Cal. for C<sub>14</sub>H<sub>12</sub>NO<sub>2</sub>Br: C, 54.87; H, 3.92; N, 4.57 %).

### 2.1.3. Ligand L<sup>3</sup> (ovan-2-tol)

The procedure was the same as for ligand L<sup>1</sup> using *o*-vanillin and 2-methylaniline (*o*-toluidine). A yellow precipitate was obtained. Yield: 1.148 g (48 %);  $\delta\text{H}$  (400 MHz, CDCl<sub>3</sub>): 13.85(1H, s, -OH); 8.64(1H, s, HC=N); 7.31(2H, t, J 8.7 Hz); 7.23(1H, d, J 8.2 Hz); 7.18(1H, d, J 7.6 Hz); 7.05(2H, m); 6.93(1H, t, J 7.5 Hz);

3.99(3H, s, -OCH<sub>3</sub>) and 2.44(3H, s, -CH<sub>3</sub>).  $\delta\text{C}$  (400 MHz, CDCl<sub>3</sub>): 162.36(HC=N); 152.12(Ar-OCH<sub>3</sub>); 148.96(Ar-N=C); 147.37(Ar-OH); 133.01(Ar-Br); 128.27, 128.14, 124.30, 119.00, 115.44(Ar-C), 56.57(-OCH<sub>3</sub>) and 18.66(-CH<sub>3</sub>); (Found: C, 74.11; H, 6.26; N, 5.68 %; Cal. for C<sub>15</sub>H<sub>15</sub>NO<sub>2</sub>: C, 74.66; H, 6.27; N, 5.80 %).

### 2.1.4. Ligand L<sup>4</sup> (ovan-2-nis)

The procedure was the same as for ligand L<sup>1</sup> using *o*-vanillin and 2-methoxyaniline (*o*-anisidine). A red precipitate was obtained. Yield: 2.30 g (89 %);  $\delta\text{H}$  (400 MHz, CDCl<sub>3</sub>): 14.42(1H, s, -OH); 8.74(1H, s, HC=N); 7.27(2H, t, J 7.3 Hz); 7.20(1H, d, J 8.5 Hz); 7.15(1H, d, J 8.2 Hz); 7.05(2H, m); 6.93(1H, t, J 8.1 Hz); 3.97(3H, s, -OCH<sub>3</sub>); and 3.93(3H, s, -OCH<sub>3</sub>).  $\delta\text{C}$  (400 MHz, CDCl<sub>3</sub>): 161.77(HC=N); 153.49, 153.08(Ar-OCH<sub>3</sub>); 136.79 (Ar-N=C); 149.19(Ar-OH); 128.42, 123.95, 119.99, 118.40, 112.42, 110.90(Ar-C); 56.57, 56.28(-OCH<sub>3</sub>). Found: C, 69.35; H, 5.98; N, 5.30 %; Cal. for C<sub>15</sub>H<sub>15</sub>NO<sub>3</sub>: C, 70.02; H, 5.88; N, 5.44 %).

### 2.1.5. Ligand L<sup>5</sup> (sal-2-cla)

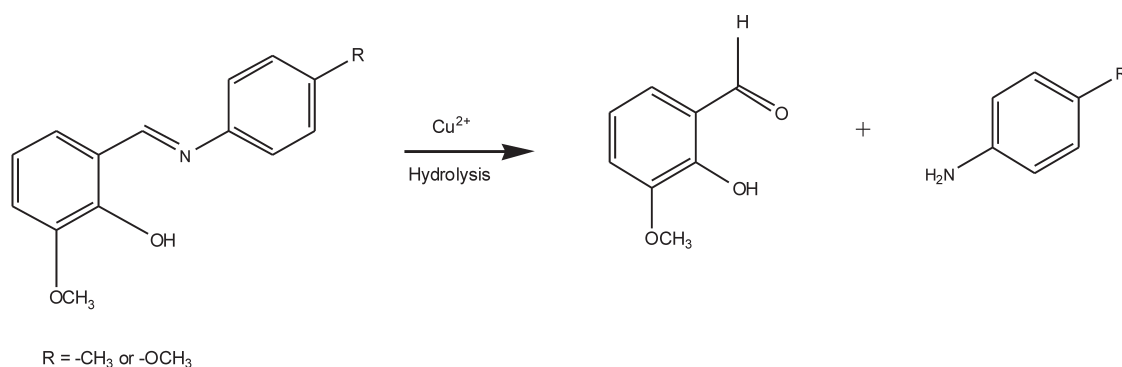
The procedure was the same as for ligand L<sup>1</sup> using salicylaldehyde and 2-chloroaniline. A yellow precipitate was obtained. Yield: 1.05 g, 45 %;  $\delta\text{H}$  (400 MHz, CDCl<sub>3</sub>): 13.21(1H, s, -OH); 8.66(1H, s, HC=N); 7.75(1H, d, J 8.5 Hz); 7.50(2H, t, J 8.2 Hz); 7.42(1H, d, J 8.0 Hz); 7.30(1H, d, J 7.4 Hz); 7.22(1H, t, J 7.8 Hz); 7.11(1H, d, J 8.5 Hz); 7.00(1H, t, J 8.0 Hz).  $\delta\text{C}$  (400 MHz, CDCl<sub>3</sub>): 163.74(Ar-OH); 161.81(HC=N); 145.86(Ar-N=C); 130.65(Ar-Cl); 134.11, 132.90, 129.95, 128.16, 128.12, 119.65, 119.54, 117.93(Ar-C); (Found: C, 66.97; H, 4.31; N, 6.00 %; Cal. for C<sub>13</sub>H<sub>10</sub>NOCl: C, 67.39; H, 4.35; N, 6.05 %).

### 2.1.6. Ligand L<sup>6</sup> (sal-2-bra)

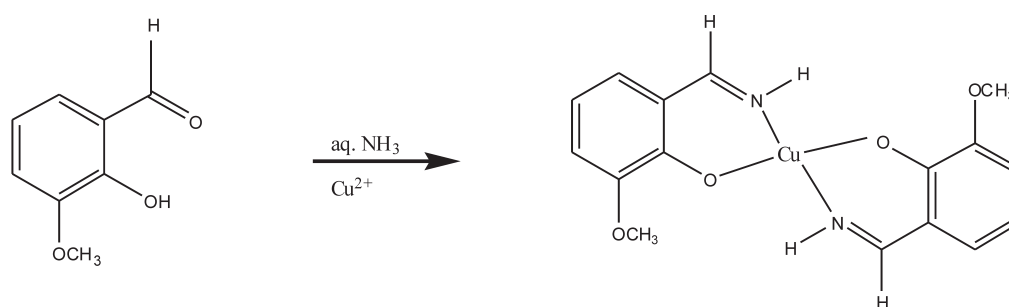
The procedure was the same as for ligand L<sup>1</sup> using salicylaldehyde and 2-bromoaniline. A yellow precipitate was obtained. Yield: 2.15 g (78 %);  $\delta\text{H}$  (400 MHz, CDCl<sub>3</sub>): 13.13(1H, s, -OH); 8.64(1H, s, HC=N); 7.71(1H, d, J 8.2 Hz); 7.45(2H, t, J 7.4 Hz); 7.39(1H, d, J 7.8 Hz); 7.27(1H, d, J 8.1 Hz); 7.18(1H, t, J 7.7 Hz); 7.09(1H, d, J 8.8 Hz); 6.99(1H, t, J 8.4 Hz).  $\delta\text{C}$  (400 MHz, CDCl<sub>3</sub>): 163.64(Ar-OH); 161.73(HC=N); 147.26(Ar-C=N); 134.13, 132.94, 128.90, 128.39, 120.37, 119.57, 119.46, 117.94(Ar-C); (Found: C, 56.43; H, 3.56; N, 5.04 %; Cal. for C<sub>13</sub>H<sub>10</sub>NOBr: C, 56.49; H, 3.62; N, 5.79 %).

### 2.1.7. Ligand L<sup>7</sup> (sal-2-tol)

The procedure was the same as for ligand L<sup>1</sup> using salicylaldehyde and *o*-methylaniline (*o*-toluidine). Yield: 1.55 g (73 %);  $\delta\text{H}$  (400 MHz, CDCl<sub>3</sub>): 13.48(1H, s, -OH); 8.62(1H, s, HC=N); 7.44 (2H, m); 7.32(2H, t, J 8.6 Hz); 7.24(1H, d, J 8.8 Hz); 7.15(1H, d, J 7.6 Hz); 7.08(1H, d, J 8.38 Hz); 6.99(1H, t, J 7.8 Hz); 2.44(3H, s, -CH<sub>3</sub>).  $\delta\text{C}$  (400 MHz, CDCl<sub>3</sub>): 162.69 (Ar-OH); 161.65 (Ar-OCH<sub>3</sub>; Ar-N=C); 147.93(Ar-OH); 133.56(Ar-CH<sub>3</sub>); 132.77, 132.64, 131.15, 127.47, 127.30, 119.79, 119.48, 118.19, 117.66(Ar-C) and 18.68(-CH<sub>3</sub>). (Found: C, 78.84; H, 6.19; N, 6.6 %; Cal. for C<sub>14</sub>H<sub>13</sub>NO: C, 79.52; H, 6.15; N, 6.63 %).



Scheme 2 Hydrolysis of the Schiff base ligands.

Scheme 3 Formation of Cu(ovan-NH)<sub>2</sub> from the hydrolysed Schiff base ligand.

### 2.1.8. Ligand L<sup>8</sup> (sal-2-nis)

The procedure was the same as for ligand L<sup>1</sup> using salicylaldehyde and *o*-methoxyaniline (*o*-anisidine). Yield: 1.395 g (61 %);  $\delta$ H (400 MHz, CDCl<sub>3</sub>): 13.41(1H, s, -OH); 8.64(1H, s, HC=N); 7.38 (2H, m); 7.29(2H, t, J 7.80 Hz); 7.20(1H, d, J 8.2 Hz); 7.10(1H, d, J 8.2 Hz); 7.05(1H, d, J 8.4 Hz); 6.90(1H, t, J 8.0 Hz); 3.88(3H, s, -OCH<sub>3</sub>).  $\delta$ C (400 MHz, CDCl<sub>3</sub>): 162.54(Ar-OH); 162.02(HC=N); 153.40(Ar-OCH<sub>3</sub>); 137.55 (Ar-N=C); 133.32, 132.39, 128.30, 121.39, 120.09, 119.95, 119.17, 117.82, 112.33(Ar-C); and 56.29(-OCH<sub>3</sub>). Found: C, 73.12; H, 5.89; N, 6.12 %. Cal. for C<sub>14</sub>H<sub>13</sub>NO<sub>2</sub>: C, 73.99; H, 5.77; N, 6.16 %

## 2.2. Synthesis of the Complexes

0.114 g (0.573 mmol) of copper acetate monohydrate, Cu(OAc)<sub>2</sub>·H<sub>2</sub>O, was dissolved in 10 mL ethanol and added dropwisely to a vigorously stirring 0.30 g (1.15 mmol) ethanolic solution of ligand L<sup>1</sup>. The resulting solution was refluxed for 6 h to obtain a brown precipitate. It was filtered under suction, washed thoroughly with ethanol and dried over silica gel in a desiccator. The same procedure was repeated for all the complexes. However, the addition of aqueous ammonia to L<sup>3</sup>/

Cu(OAc)<sub>2</sub>·H<sub>2</sub>O aliquot resulted in the hydrolysis of the imine bond and consequent formation of an ammonia-based complex as shown in Schemes 2 and 3. The same product was obtained with ligand L<sup>4</sup>. The analytical and the spectral data for the complexes are presented in Tables 1 and 2.

## 3. X-ray Crystallography Data

Single crystals of the [Cu(ovan-NH)<sub>2</sub>].H<sub>2</sub>O complex were obtained *via* slow evaporation of the saturated DMF solution of the complex. A suitable single crystal of the complex was diffracted using a Bruker KAPPA APEX II single crystal x-ray diffractometer, with a 4-circle Kappa goniometer. The crystallographic data was collected at 200 K and 0.7107 nm (III) on a sensitive CCD detector with graphite-monochromated MoK $\alpha$  radiation, a total of 1978 reflections was collected of which 1836 were considered observed [ $I > 2\sigma(I)$ ]. The structure was solved by direct method using the program SHELXL-97<sup>28</sup> and refined anisotropically by full matrix least-squares on F<sup>2</sup> using SHELXL-97. The details of crystallographic parameters, data collection and refinements are listed in Table 3 while selected bond lengths and angles are presented in Table 4.

Table 1 Physical and analytical data for the complexes.

Complexes	Colour	% Yield	M. Pt/°C	Molar mass	% Found (calculated)				$\Lambda_M$ /Ω <sup>-1</sup> cm <sup>2</sup> mol <sup>-1</sup>
					C	H	N	Cu	
[Cu(L <sup>1</sup> ) <sub>2</sub> ] <sup>*</sup>	Brown	60	174–176	584.85	57.48 (57.50)	3.80 (3.79)	4.71 (4.79)	10.50 (10.87)	2.90 <sup>a</sup> DMF
[Cu(L <sup>2</sup> ) <sub>2</sub> ]	Brown	68	172–174	673.91	49.97 (49.90)	3.36 (3.29)	4.12 (4.16)	(9.57) (9.43)	6.59 <sup>a</sup> DMF
[Cu(ovan-NH) <sub>2</sub> ].H <sub>2</sub> O	Green	91	166–168	399.90	47.70 (48.06)	5.07 (5.04)	6.97 (7.01)	15.80 (15.89)	1.80 <sup>a</sup> DMF
[Cu(L <sup>5</sup> ) <sub>2</sub> ]	Brown	81	250–252	524.93	59.24 (59.49)	3.36 (3.46)	5.29 (5.34)	12.30 (12.10)	1.65 <sup>a</sup> DMF
[Cu(L <sup>6</sup> ) <sub>2</sub> ]	Brown	76	248–249	613.83	51.92 (51.87)	2.83 (2.96)	4.51 (4.56)	10.60 (10.35)	1.59 <sup>a</sup> DMF
[Cu(L <sup>7</sup> ) <sub>2</sub> ]	Green	91	230–232	484.09	70.52 (69.47)	5.11 (5.00)	5.73 (5.76)	12.30 (12.10)	2.71 <sup>a</sup> DMF
[Cu(L <sup>8</sup> ) <sub>2</sub> ]	Brown	76	208–210	516.07	64.66 (65.16)	4.74 (4.69)	5.38 (5.43)	12.0 (12.31)	4.65 <sup>a</sup> DMF

<sup>\*</sup>L<sup>1</sup> = ovan-2-cla; L<sup>2</sup> = ovan-2-bra; L<sup>5</sup> = sal-2-cla; L<sup>6</sup> = sal-2-bra; L<sup>7</sup> = sal-2-tol; L<sup>8</sup> = sal-2-nis. <sup>a</sup>Neutral complex

**Table 2** Infrared and UV-visible spectral data for the Schiff base ligands and the complexes.

Compounds	$\nu_{\text{OH}}/\text{cm}^{-1}$	$\nu_{\text{C=N}}/\text{cm}^{-1}$	$\nu_{\text{C=O}}/\text{cm}^{-1}$	$\nu_{\text{Cu-O}}/\text{cm}^{-1}$	$\nu_{\text{Cu-N}}/\text{cm}^{-1}$	$\lambda_{\text{max}}/\text{nm}$
L <sup>1</sup>	3149–2365	1613	1277	–	–	215, 227, 284, 320, 471
Cu(L <sup>1</sup> ) <sub>2</sub>	–	1603	1336	559	464	236, 292, 403, 661
L <sup>2</sup>	3114–2370	1613	1277	–	–	211, 228, 273, 325, 344
Cu(L <sup>2</sup> ) <sub>2</sub>	–	1604	1331	561	460	235, 292, 404, 665
L <sup>3</sup>	3104–2349	1612	1277	–	–	212, 225, 276, 320, 455
Cu(ovan-NH) <sub>2</sub> .H <sub>2</sub> O**	3413, 3294	1623	1331	541	482	237, 274, 378, 654
L <sup>4</sup>	3104–2344	1614	1280	–	–	216, 229, 280, 346, 462
Cu(ovan-NH) <sub>2</sub> .H <sub>2</sub> O**	3413, 3294	1623	1331	541	482	237, 274, 378, 654
L <sup>5</sup>	3220–2359	1612	1273	–	–	209, 227, 272, 324, 342
Cu(L <sup>5</sup> ) <sub>2</sub>	–	1603	1325	556	455	213, 238, 398, 667
L <sup>6</sup>	3220–2359	1612	1277	–	–	214, 227, 285, 319, 470
Cu(L <sup>6</sup> ) <sub>2</sub>	–	1602	1325	556	466	226, 281, 393, 663
L <sup>7</sup>	3104–2334	1615	1279	–	–	208, 224, 266, 337, 442
Cu(L <sup>7</sup> ) <sub>2</sub>	–	1606	1325	575	449	229, 373, 394, 657
L <sup>8</sup>	3084–2284	1615	1280	–	–	205, 228, 268, 346, 450
Cu(L <sup>8</sup> ) <sub>2</sub>	–	1606	1329	580	457	216, 283, 390, 683

\*\*Cu(ovan-NH)<sub>2</sub>.H<sub>2</sub>O was prepared using aqueous ammonia.

## 4. Results and Discussion

### 4.1. Elemental Analysis and Conductivity Measurement

The elemental analysis results for the complexes suggest that the complexes are of the form CuL<sub>2</sub>, with the Cu<sup>2+</sup> ion coordinating to the Schiff base ligands in a ratio 1:2. The low conductivity values for the complexes imply the Schiff base ligands coordinated as neutral species<sup>29</sup> via the deprotonated phenolic oxygen-O, and the imine nitrogen-N, atoms.

### 4.2. <sup>1</sup>H- and <sup>13</sup>C- NMR

The assignments of the main NMR signals are given in the experimental section. The azomethine proton (HC=N) absorbed downfield in all the Schiff base ligands as one proton singlet at 8.64–8.68 ppm while the broad signal at 13.82–13.13 ppm is assigned to the phenolic hydroxyl proton. The position of the hydroxyl signal is attributable to a strong intramolecular hydrogen bonding through the imine -N and the

phenolic -O. The signal at 163.51–160.88 ppm is due to the imine functional group (C=N), and further confirms the formation of the Schiff base ligands. The purity of the ligands was, however, indicated by the disappearance of the amino and aldehyde protons at 3–4 ppm and 9–10 ppm, respectively. The methoxy substituent of the *o*-vanillin analogues was observed as a strong singlet at 3.99–3.97 ppm. In addition, the signals at 2.44 ppm and 3.93–3.88 ppm correspond to the methyl and methoxy substituents of the toluidine and anisidine-based ligands, respectively.

### 4.3. Infrared Study

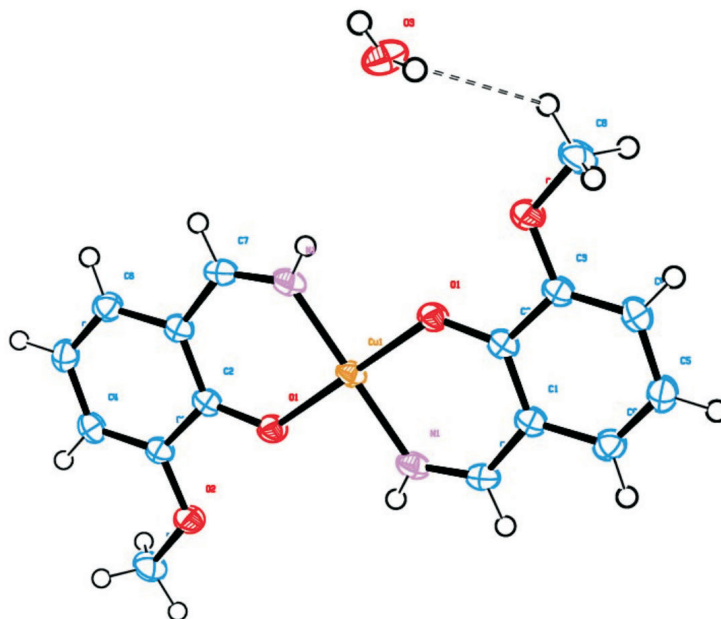
The imine (C=N) functional group of the free ligands was observed as a strong band at 1615–1612 cm<sup>-1</sup>. As expected, the band red-shifted in the spectra of the complexes, except [Cu(ovan-NH)<sub>2</sub>].H<sub>2</sub>O, indicating coordination of the Schiff base ligands via the azomethine N-atom.<sup>24,26,30–31</sup> In addition, the strong band at 1280–1273 cm<sup>-1</sup> corresponds to the phenolic C-O stretching vibration of the free ligands. This band blue-shifted to 1336–1325 cm<sup>-1</sup> upon complexation,<sup>5,31–32</sup> suggesting the involvement of the phenolic oxygen atom in the coordination sphere. All the infrared spectra of the free ligands were characterized by a broad band at 3220–2284 cm<sup>-1</sup> corresponding to the O-H stretching vibration of the ligands. The broadness was due to a strong intramolecular hydrogen bonding between the imine nitrogen and the hydroxyl group.<sup>33–34</sup> The hydroxyl band, however, disappeared in the spectra of the complexes due to deprotonation and involvement of the oxygen atom in the coordination sphere. The mode of coordination of the Schiff base

**Table 3** Crystal data and structure refinements for the [Cu(ovan-NH)<sub>2</sub>].H<sub>2</sub>O complex.

Empirical formula	C <sub>16</sub> H <sub>20</sub> CuN <sub>2</sub> O <sub>6</sub>
Formula weight	399.88
Temperature/K	200(2)
Wavelength/Å	0.71073
Crystal system	Monoclinic
Space group	P 2 <sub>1</sub> /c
a/Å	10.9309 (3)
b/Å	4.85600 (10)
c/Å	17.7030 (5)
V/Å <sup>3</sup>	816.58 (4)
Z	2
D/g cm <sup>-3</sup>	1.06124
$\mu/\text{mm}^{-1}$	1.374
F (000)	414
$\Theta$ range for data collection/°	2.24–28.00
No. reflections/observed	1978/1836
Data/restraints/parameter	1978/0/135
Goodness-of-fit	1.080
Refinement method	Full-matrix least squares of F <sup>2</sup>
Final R indices [F > 4 $\sigma$ (F)]	R1 = 0.0204, WR2 = 0.0574
R indices (all data)	R1 = 0.0225, WR2 = 0.0593

**Table 4** Selected bond lengths and bond angles for [Cu(ovan-NH)<sub>2</sub>].H<sub>2</sub>O.

Bond lengths	Å	Bond angles	°
Cu1-O1	1.9447(9)	O1-Cu1-N1	92.19(5)
Cu1-N1	1.915(2)	O1-Cu1-O1	180.00(4)
N1-H1	0.78(2)	O1-Cu1-N1	87.81(5)
N1-C7	1.279(2)	N1-Cu1-O1	87.81(5)
O1-C2	1.305(2)	N1-Cu1-N1	180.00(6)
O2-C3	1.3729(2)	O1-Cu1-N1	92.19(5)
O2-C8	1.423(2)	O1-C2-C1	125.5(1)
O3-H3A	0.79(2)	O1-C2-C3	118.2 (1)
O3-H3B	0.73(3)		



**Figure 1** ORTEP diagram for  $[\text{Cu}(\text{ovan-NH})_2]\cdot\text{H}_2\text{O}$  with limited labelling for clarity, showing 50 % probability ellipsoids.

ligand was further substantiated by the appearance of two new bands in the far infrared spectra of the complexes at  $580\text{--}541\text{ cm}^{-1}$  and  $482\text{--}449\text{ cm}^{-1}$ . These bands are assigned to the  $\nu_{\text{Cu-O}}$  and  $\nu_{\text{Cu-N}}$  stretches, respectively.<sup>4,5,33</sup>

Furthermore, the infrared spectrum of  $\text{Cu}(\text{ovan-NH})_2$  has two additional strong bands at  $3413\text{ cm}^{-1}$  and  $3294\text{ cm}^{-1}$ . The bands are attributed to the N-H stretching vibrations of ligand  $\text{ovan-NH}$  that was obtained upon the addition of aqueous ammonia to the aliquot of  $\text{L}^3/\text{L}^4$  and  $\text{Cu}(\text{OAc})_2\cdot\text{H}_2\text{O}$  as shown in Scheme 3.

#### 4.4. UV/Visible Study

The electronic transition study of the compounds was carried out in methanol. Three absorption bands were observed in the spectra of the Schiff base ligands at  $229\text{--}211\text{ nm}$ ,  $292\text{--}268\text{ nm}$  and  $394\text{--}319\text{ nm}$ . The bands at higher energy values ( $229\text{--}211\text{ nm}$ ) are attributed to the  $\pi \rightarrow \pi^*$  transition of the benzene ring<sup>3</sup> while the other two bands are assigned to the  $\pi \rightarrow \pi^*$  and  $n \rightarrow \pi^*$  transitions of the azomethine chromophore, respectively.<sup>3,12</sup> These transitions are observed at a lower energy values in the spectra of the complexes, indicating coordination *via* the azomethine functional group ( $\text{HC}=\text{N}$ ).

The  $d \rightarrow d$  transition of the copper(II) ion was observed as a broad band at  $683\text{--}654\text{ nm}$ , indicating a square planar geometry. Bhunora *et al.* observed similar band at  $662\text{--}628\text{ nm}$  for a series of square planar salicyaldiminecopper(II) complexes.<sup>26</sup> The Schiff base ligands showed an additional absorption band at above  $400\text{ nm}$  ( $470\text{--}442\text{ nm}$ ) in methanol, and this new band has been previously assigned to the keto-imine form of ortho-hydroxy-salicyaldimines in polar and non-polar solvents.<sup>3</sup> The tautomerism is thought to occur *via* an intramolecular (or intermolecular in protic solvents) hydrogen transfer to the imine nitrogen.

#### 4.5. Molecular Structure of $[\text{Cu}(\text{ovan-NH})_2]\cdot\text{H}_2\text{O}$

The labelled ORTEP diagram of  $[\text{Cu}(\text{ovan-NH})_2]\cdot\text{H}_2\text{O}$  is presented in Fig. 1 while the bond lengths and angles are presented in Table 4. The crystal structure consists of two units of the Schiff base ligands chelating with the copper(II) ion in a planar milieu. The copper(II) ion crystallizes in the monoclinic system with a space group of  $\text{P}21/\text{c}$  having  $a = 10.9309$ ,  $b = 4.85600$ ,  $c = 17.7030$ ,  $\alpha = \gamma = 90^\circ$  and  $\beta = 119.658^\circ$ . The bidentate Schiff

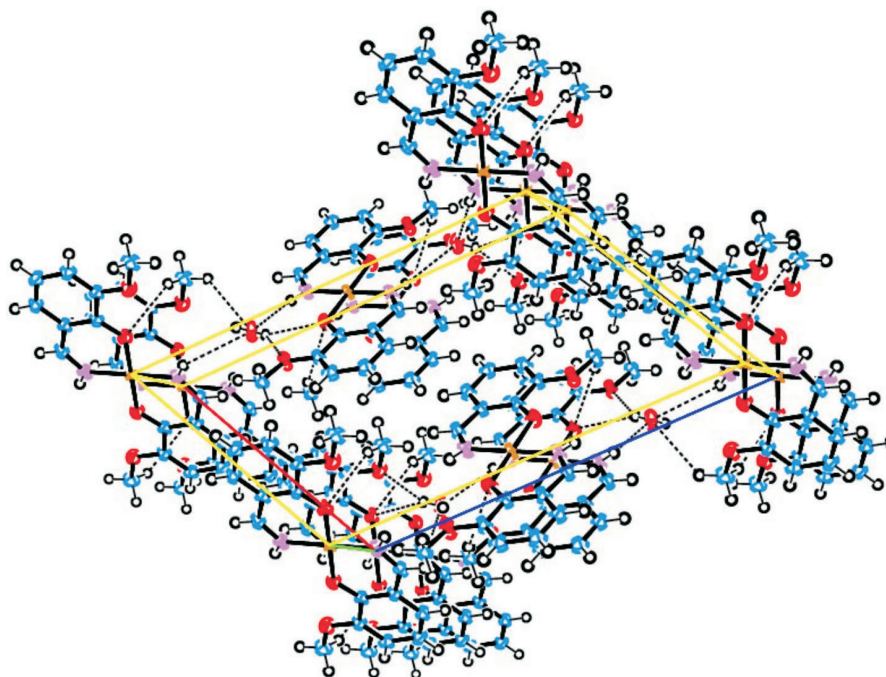
base ligand formed a four coordinate chelate with the copper(II) ion in a trans configuration through the imine nitrogen and the phenolic oxygen atoms. The geometry is slightly distorted from planarity with  $\text{O1-Cu1-N1}$  bond angles of  $92.19^\circ$  and  $87.8^\circ$ . The bond lengths for the  $\text{Cu-O}$  ( $1.9447\text{ \AA}$ ) and  $\text{Cu-N}$  ( $1.915\text{ \AA}$ ) fall within the range of reported values for similar structures in the literature ( $1.999$  and  $1.869$ ;  $1.8959$  and  $1.9769$ ;  $1.901$  and  $1.9769\text{ \AA}$ ).<sup>26</sup> The structures reported by Bhunora *et al.*,<sup>26</sup> equally have the copper(II) ion in a planar geometry within the trans configuration of the  $\text{N}_2\text{O}_2$  donor atoms. In addition, there exist intra-molecular hydrogen bonding with average bond length ( $\text{O-H}$ )  $0.76\text{ \AA}$  (Fig. 2), occasioned by the presence of a water molecule in the crystal lattice of the complex.

#### 5. Biological Study

The Schiff base ligands and their respective copper(II) complexes were screened for their *in vitro* antibacterial and antifungal activity against *Escherichia coli* ATCC® 8739™\*, *Staphylococcus aureus* subsp. *aureus* ATCC® 6538™\*, *Bacillus subtilis* subsp. *spizizenii* ATCC® 6633™\* and *Candida albicans* ATCC® 2091™\*.

#### 5.1. Disc Diffusion Technique

The qualitative antimicrobial susceptibility testing of the compounds was evaluated using the disc diffusion technique.<sup>34</sup> Each test organism was inoculated onto a nutrient agar plate and incubated at  $37^\circ\text{C}$  for 24 h to obtain the primary culture. Several discrete colonies were picked from the culture to make a bacterial suspension ( $10\text{ mL}$ ) in a test tube using saline water. The turbidity of the suspension was compared with 0.5 McFarland standard to obtain  $10^6\text{--}10^8\text{ CFUs}$ . The bacterial suspension ( $0.1\text{ mL}$ ) was inoculated onto Mueller Hinton plate and the sterile discs that have been impregnated with the test compounds were firmly placed on it. The assay was inoculated at  $37^\circ\text{C}$  for 16 h and the zone of inhibition was measured as millimetres diameter. Ampicillin was used as the standard antibacterial drug while the control solvent was dimethylformamide (DMF). The test was repeated two more times for those compounds that showed activity of more than 6.5 mm, and their activity was recorded as average zone of inhibition in Table 5. Similar procedure was repeated for the antifungal susceptibility testing of the com-



**Figure 2** Chains of hydrogen bonding in the crystal lattice of  $[\text{Cu}(\text{ovan-NH}_2)_2]\cdot\text{H}_2\text{O}$  viewed along the b-axis.

pounds using the potato disc assay instead of Mueller Hinton agar. The standard antifungal drug used for the study was ketoconazole.

## 5.2. Minimum Inhibitory Concentration (MIC)

The quantitative antimicrobial activity of the test compounds was evaluated using micro dilution broth method according to Clinical and Laboratory Standard Institute (formally NCCLS).<sup>34</sup> Two-fold serial dilutions of the compounds were prepared in 96 microwell plates using sterile nutrient broth as the diluent. The plates were inoculated with 5  $\mu\text{L}$  bacterial suspensions containing  $10^6$ – $10^8$  CFUs and incubated at 37 °C for 16–18 h. The MIC value was defined as the lowest concentration of the

**Table 5** Diameters of zone of inhibition (mm) for the Schiff base ligands and the complexes.

No.	Compounds	Gram-positive		Gram-negative	Fungus
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>C. albicans</i>
1	L <sup>1</sup> (ovan-2-cla)	12	–	8.5	20
2	Cu(L <sup>1</sup> ) <sub>2</sub>	–	–	–	16
3	L <sup>2</sup> (ovan-2-bra)	12	–	09	21
4	Cu(L <sup>2</sup> ) <sub>2</sub>	–	08	07	16
5	L <sup>3</sup> (ovan-2-tol)	11.5	10	16	26
6	[Cu(ovan-NH) <sub>2</sub> ]	ns*	ns*	ns*	ns*
7	L <sup>4</sup> (ovan-2-nis)	16	16	14	44
8	[Cu(ovan-NH) <sub>2</sub> ]	ns*	ns*	ns*	ns*
9	L <sup>5</sup> (sal-2-cla)	–	08	–	08
10	Cu(L <sup>5</sup> )	ns*	ns*	ns*	ns*
11	L <sub>VII</sub> (sal-2-bra)	–	08	–	–
12	Cu(L <sup>6</sup> )	ns*	ns*	ns*	ns*
13	L <sup>7</sup> (sal-2-tol)	–	08	08	07
14	Cu(L <sup>7</sup> )	–	–	–	–
15	L <sup>8</sup> (sal-2-nis)	–	09	–	07
16	Cu(L <sup>8</sup> )	07	–	08	16
	Ampicillin	52	38	28	
	Ketoconazole				20
	DMF	–	–	–	–

Note: disc diameter is 6.5 mm; \*ns: not soluble; (–): not active.

compounds giving complete inhibition of visible growth. The MIC values for the compounds varied from 6.250–0.1953 mg/mL and the result is presented in Table 6.

The diameters of the zone of inhibition and the minimum inhibitory concentrations (MIC) of the compounds are presented in Tables 5 and 6, respectively. The compounds were not as active as penicillin, but showed significant antifungal activity against the tested organisms.

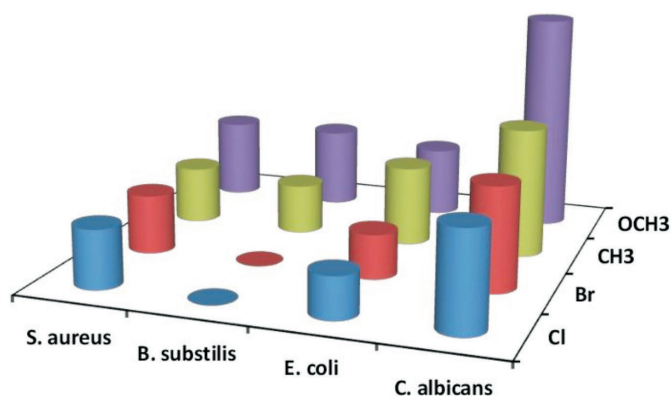
The *o*-vanillin-based ligands exhibited higher activity than the salicylaldehyde-based compounds which were virtually non-active against the tested organisms. This could be due to the presence of the methoxy group at the ortho position of the aldehyde moiety of the Schiff base ligands. Likewise, ligands L<sup>3</sup> and L<sup>4</sup> (toluidine and anisidine derivatives) showed significant potency against the microorganisms (Fig. 3); thus relating the activity of the Schiff bases to the electronic effects of the substituents, since the chloro- and the bromo- analogues exhibited only slight activity. Ansari *et al.*<sup>35</sup> has, however, reported high antimicrobial activity for the *o*-chloro, *o*-methyl, *o*-methoxyl and *p*-amino analogues in a series of substituted benzimidazole Schiff base ligands.

On chelation, however, the presence of the Cu(II) ions did not

**Table 6** MIC values for the Schiff base ligands and the complexes ( $1 \times 10^{-1}$  mg/mL).

No.	Compounds	Gram-positive		Gram-negative
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>
1	L <sup>1</sup>	1.9531 (12)	–	1.9531 (8.5)
2	L <sup>2</sup>	7.8125 (12)	–	7.8125 (09)
3	CuL <sup>2</sup>	–	7.8125 (08)	62.50 (07)
4	L <sup>3</sup>	3.9063 (11.5)	7.8125 (10)	1.9531 (16)
5	L <sup>4</sup>	3.9063 (16)	3.9063 (16)	3.906 (14)
6	L <sup>5</sup>	–	31.25 (08)	–
7	LVI	–	31.25 (08)	–
8	LVII	–	15.625 (08)	7.8125 (08)
9	LVIII	–	15.625 (09)	–
10	Cu(LVIII) <sub>2</sub>	31.250 (07)	–	31.250 (08)

Note: the diameter of zone of inhibition is given in brackets.



**Figure 3** Antimicrobial activity of the *o*-vanillin derivatives of the Schiff base ligands.

enhance the antimicrobial activity of the free ligands. The copper complexes exhibited lower activity than the Schiff base ligands. Though, it is believed that chelation increases the lipophilicity of the metal ion and consequently increases the antimicrobial activity of metal complexes relative to the free ligands. Conversely, it has been observed that the free ligands do possess higher activity in some cases; an indication that activity does not depend solely on the presence of metal ions but rather a synergistic effect of many factors. Thus, understanding the mechanisms of action of potential antimicrobial agent is considered a critical aspect of our study for the future.

## 6. Conclusion

The ortho-substituted Schiff bases coordinated as monobasic bidentate ligands *via* the imine nitrogen and phenolic oxygen atoms. The crystal structure of bis(3-methoxy-2-methylimine) copper(II) complex, indicated a planar configuration for the complexes. The antimicrobial study of the compounds revealed higher activity for the *o*-vanillin derivatives than the salicylaldehyde-based ligands.

## 7. Supplementary Data

X-ray crystallography data of bis(3-methoxy((imine)methylphenol)Cu(II) complex has been deposited with the Cambridge Crystallographic Data Centre (CCDC) and can be obtained free of charge on request at <http://www.ccdc.cam.ac.uk/conts/retrieving.html> or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0)1223-336033; e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk), quoting the CCDC number, 888472.

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