

# Synthesis, crystal structures, spectroscopic characterization and *in vitro* antidiabetic studies of new Schiff base Copper(II) complexes

SUNDARAMURTHY SANTHA LAKSHMI<sup>a</sup>, KANNAPPAN GEETHA<sup>b,\*</sup>, M GAYATHRI<sup>c</sup> and GANESH SHANMUGAM<sup>d</sup>

<sup>a</sup>Department of Chemistry, D K M College for Women, Vellore, Tamil Nadu, 632 001, India

<sup>b</sup>Department of Chemistry, Muthurangam Govt. Arts College, Vellore, Tamil Nadu, 632 002, India

<sup>c</sup>Department of Biotechnology, School of Bio-Sciences and Technology, VIT University, Vellore, Tamil Nadu, 632 014, India

<sup>d</sup>Bioorganic Chemistry Laboratory, CSIR-Central Leather Research Institute, Adyar, Chennai, Tamil Nadu, 600 020, India

e-mail: santhalakshmi\_s@yahoo.com; geethamgacchem@gmail.com; gayathrigopinath@vit.ac.in; ganesh@clri.res.in

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**Abstract.** Two new Schiff base copper(II) complexes, [CuL<sup>1</sup>(tmen)] (**1**) and [Cu<sub>2</sub>L<sub>2</sub><sup>2</sup>(tmen)] (**2**) {where, H<sub>2</sub>L<sup>1</sup> = N-(salicylidene)-L-valine, H<sub>2</sub>L<sup>2</sup> = N-(3,5-dichlorosalicylidene)-L-valine and tmen = N,N,N',N'-tetramethylethylene-1,2-diamine} have been synthesized and characterized by molar conductance, elemental analyses, VSM-RT, UV-Vis, FTIR, EPR, and CD spectra. Both the complexes were structurally characterized by single crystal XRD. The crystal structure of complex **1** displays a distorted square pyramidal geometry in which Schiff base is coordinated to the Cu(II) ion *via* ONO-donor in the axial mode, whereas, the chelating diamine displays axial and equatorial mode of binding *via* NN-donor atoms. The crystal structure of the complex **2** reveals a *syn-anti* mode of carboxylate bridged dinuclear complex, in which, the coordination geometry around Cu(1) is square pyramid and distorted square planar around Cu(2). The target complexes were screened for *in vitro* antidiabetic activity. Both the complexes showed good inhibitory activity for  $\alpha$ -amylase and  $\alpha$ -glucosidase.

**Keywords.** Schiff base; Copper(II) complexes; crystal structure; *syn-anti* mode; antidiabetic.

## 1. Introduction

Diabetes mellitus is an endocrine disorder, which causes 9% of deaths worldwide. A survey reported that diabetes mellitus is affecting 10% of the population every year in developing countries.<sup>1</sup> WHO predicted that the morbidity and mortality rate is increasing rapidly due to diabetes.<sup>2</sup> To prevent this death rate many medications are used, however they failed to alleviate the complications. Treating diabetes with metal complexes is a new therapeutic strategy. Coulson and Dandona reported that ZnCl<sub>2</sub> stimulate lipogenesis in rat adipocytes, similar to the action of insulin.<sup>3</sup> The transition metal complexes were described to exhibit insulin mimetic effect as well as  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition activity.<sup>4</sup> Copper is one of the transition metal found in heart, lungs, liver and gall bladder. Copper is essential to maintain the normal structure and function

of cells, and required for survival and growth. It plays a significant role in electron transfer reactions<sup>5</sup> and involved in an iron metabolism, immune system, normal metabolism of glucose and cholesterol. Decrease in copper level results in oxidative damage and other metabolic changes.<sup>6</sup> Various studies have been conducted on copper complexes and proved their different pharmacological actions such as antiulcer, anticonvulsant, anticancer, antidiabetic and antimicrobial activity.<sup>7–10</sup>

Since interdisciplinary research is a milestone of coordination chemistry, many researchers focus on the areas like catalysis, photochemistry, molecular modelling and biological studies.<sup>11</sup> Until now, amino acid Schiff base copper(II) complexes containing NN-donor heterocyclics like 1,10-phenanthroline, 2,2'-bipyridine have been reported,<sup>12,13</sup> whereas, use NN-donor aliphatic compounds was not yet reported. Similarly, most of the reported ternary Cu(II) complexes containing Schiff bases and heterocyclic compounds were analysed for antimicrobial, antitumor and antioxidant activities.<sup>14,15</sup>

\*For correspondence

The complexes derived from Schiff bases,<sup>16,17</sup> especially those of salicylidene amino acids, have potential biological properties.<sup>18</sup> Vančo *et al.*,<sup>19</sup> reported *in vivo* antidiabetic activity of Cu(II) and Zn(II) complexes derived from N-salicylidene- $\beta$ -alanine. Herein, we report the synthesis of new Schiff base Cu(II) complexes **1** and **2** containing NN-donor aliphatic chelating diamine, tmen (N,N,N',N'-tetramethylethylene-1,2-diamine) and study of the *in vitro* antidiabetic activity. According to the literature, electron withdrawing substituents present in the phenyl ring of the Schiff bases show very good antimicrobial and other biological properties.<sup>20,21</sup> Hence, we have chosen chloro substituent in case of H<sub>2</sub>L<sup>2</sup>.

## 2. Experimental

### 2.1 Chemicals and physical measurements

All the reagents and chemicals were procured from commercial sources and were used as received. L-valine was obtained from SD Fine chemicals, Mumbai. Salicylaldehyde and 3,5-dichlorosalicylaldehyde were purchased from Sigma Aldrich and tmen (N,N,N',N'-tetramethylethylene-1,2-diamine) was purchased from Central Drug House (P) Ltd, Mumbai. Chemicals such as  $\alpha$ -amylase,  $\alpha$ -glucosidase, soluble starch, glucose and DMSO were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai. Dialysis membrane and 3,5-dinitro salicylic acid (DNSA) were purchased from Himedia Laboratories, Mumbai.

Elemental analyses (C, H and N) of the complexes were performed on Flash 2000 organic elemental analyzer. Magnetic moment of both the complexes was obtained using a VSM Lake Shore-7404 at room temperature and diamagnetic corrections were made using Pascal's constants. The molar conductance of the complexes were measured in DMF (10<sup>-3</sup> M) solution using a direct reading conductivity meter at room temperature. The UV-Visible spectra of both the complexes (0.05 mg/mL) were recorded in ethanol, in the region of 200-800 nm using a Perkin-Elmer Lambda EZ201 UV-Vis spectrophotometer at room temperature. A rectangular quartz cell with path length of 1 cm was used. Circular dichroism spectra were measured using JASCO J-715 spectropolarimeter at room temperature. CD spectra of both the complexes (0.25 mg/mL) were recorded in ethanol, in the wavelength range of 200-800 nm. A quartz cell with a path length of 1 mm was used. Each spectrum represents an average of three individual scans. FTIR spectra of the complexes were recorded in the region of 4000-400 cm<sup>-1</sup> on IR Affinity-1S Shimadzu FTIR spectrophotometer using the conventional

KBr pellet methodology. The EPR spectra of the complexes were recorded in the polycrystalline state on Bruker EPR spectrometer (EMX-10/2.7) using DPPH as g-marker at liquid nitrogen temperature.

### 2.2 Synthesis

Complex **1** was synthesized by adding an aqueous solution of L-valine (1 mmol, 0.12 g) and potassium hydroxide (2 mmol, 0.12 g) to an ethanolic solution (25 mL) of salicylaldehyde (1 mmol, 0.1 mL) and the mixture was stirred at 333 K for 1 h. Then, hydrated copper(II) acetate (1 mmol, 0.201 g) was added and the solution was stirred for an hour at 333 K followed by addition of tmen (1 mmol, 0.1 mL) in drops and the solution was stirred for additional 2 h at the same temperature. The resultant green coloured turbid solution was filtered and kept at room temperature to evaporate the solvent. Similarly, complex **2** was prepared in the same way as complex **1** using 3,5-dichlorosalicylaldehyde (1 mmol, 0.195 g) instead of salicylaldehyde.

The analytical data of the complexes are given below.

Complex **1**: M.P.: 182°C;  $\mu_{\text{eff}} = 1.76$  B.M.;  $\Lambda_{\text{M}} = 3 \Omega^{-1}\text{cm}^2\text{mol}^{-1}$ ; Elemental analysis (% Found/calculated) for C<sub>18</sub>H<sub>29</sub>CuN<sub>3</sub>O<sub>3</sub>: C: 55.22/54.19; H: 7.35/7.33; N: 10.51/10.53. Complex **2**: M.P.: 204°C;  $\mu_{\text{eff}} = 1.58$  B.M.;  $\Lambda_{\text{M}} = 3.5 \Omega^{-1}\text{cm}^2\text{mol}^{-1}$ ; Elemental analysis (% Found /calculated) for C<sub>30</sub>H<sub>38</sub>Cl<sub>4</sub>Cu<sub>2</sub>N<sub>4</sub>O<sub>6</sub>: C: 43.98/43.97; H: 4.69/4.67; N: 6.85/6.84.

### 2.3 X-ray crystal structure determination

Single crystals suitable for X-ray diffraction study for the complex **1** were grown by slow evaporation of the DMF solution at room temperature, whereas for complex **2**, it was obtained by the slow evaporation of the solvent from the mother liquor at room temperature. Single crystal diffraction data of the complexes were collected on Bruker single crystal Kappa Apex II diffractometer equipped with graphite monochromatized Mo-K $\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ). A green coloured crystal of the complex **1** having the size 0.2  $\times$  0.25  $\times$  0.25 mm (and for complex **2** of the size 0.3  $\times$  0.28  $\times$  0.25 mm) was mounted on a glass fibre and was used for data collection.

The structure of the complexes was solved by direct methods, using SHELXS-97 and refined by full-matrix least-squares techniques against F<sup>2</sup> with anisotropic thermal parameters for all non-hydrogen atoms using SHELXL-97.<sup>22</sup> A summary of pertinent crystal data along with further details of structure determination and refinement is given in table S1, Supporting

information. Mercury 3.7 version was used to present the ORTEP view of the crystal structures.

**2.3a Crystal data:** Complex **1**:  $C_{18}H_{29}CuN_3O_3$ , F. Wt. = 398.98, tetragonal, space group  $P4_3$ ,  $a = 10.627(5)$ ,  $b = 10.627(5)$ ,  $c = 18.048(5)$  Å and  $\alpha = \beta = \gamma = 90^\circ$ ,  $V = 2038.2(15)$  Å<sup>3</sup>,  $Z = 4$ ,  $\rho = 1.300$  g/cm<sup>3</sup>,  $F(000) = 844$ ,  $\theta$  range = 1.92 to 28.33°, Final  $R1 = 0.0397$ ,  $wR2 = 0.0934$ .

Complex **2**:  $C_{30}H_{38}Cl_4Cu_2N_4O_6$ , F. Wt. = 819.52, Orthorhombic, space group  $P2_12_12_1$ ,  $a = 11.8583(2)$ ,  $b = 14.6280(2)$ ,  $c = 20.1371(3)$  Å and  $\alpha = \beta = \gamma = 90^\circ$ ,  $V = 3493.05(9)$  Å<sup>3</sup>,  $Z = 4$ ,  $\rho = 1.558$  g/cm<sup>3</sup>,  $F(000) = 1680.0$ ,  $\theta$  range = 2.65 to 29.24°, Final  $R1 = 0.0410$ ,  $wR_2 = 0.1107$ .

## 2.4 In vitro antidiabetic activity

Glucose diffusion inhibition assay and percentage inhibition of the enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase were carried out in DMSO by previously optimized procedure.<sup>23</sup> Standard drug acarbose was used as positive control for calculating  $IC_{50}$  values.

All the values are represented as Mean  $\pm$  SEM of triplicates. Microsoft office excel was used to calculate SEM and  $IC_{50}$  were calculated by GraphPad Prism 5.0 version. Values are considered significant with  $p \leq 0.05$ .

## 3. Results and Discussion

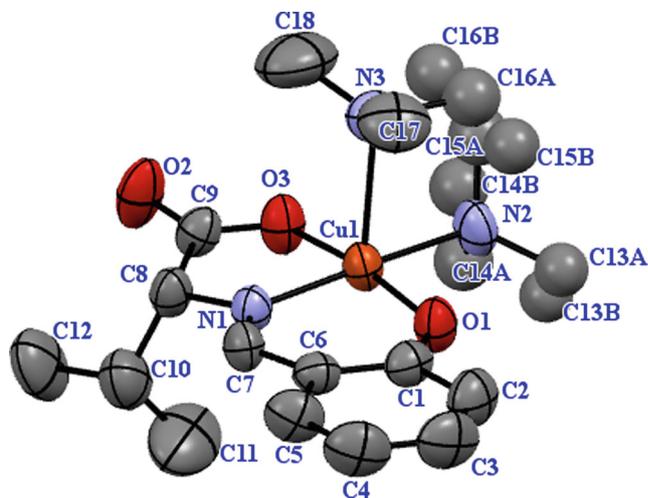
Both the complexes are stable and readily soluble in solvents such as  $CHCl_3$ , DMSO and DMF at room temperature. To study the electrolytic nature of the complexes, molar conductivities were measured in DMF at  $10^{-3}$  M. The lower molar conductivity values of 3 and 3.5  $\Omega^{-1}cm^2mol^{-1}$  for the complexes **1** and **2**, respectively, substantiate the non-electrolytic nature.<sup>24</sup>

### 3.1 X-ray crystallography

A summary of pertinent selected bond lengths and bond angles for the complexes **1** and **2** are given in table S2, in Supporting information.

**3.1a Structure description of complex 1:** An ORTEP view of the complex **1** along with the atom numbering scheme is shown in figure 1.

In general, Cu(II) complexes exist with either square pyramidal (spy) or trigonal bipyramidal (tbp) geometry in case of pentadentate coordination. Some of the Cu(II) complexes have been found to possess intermediate

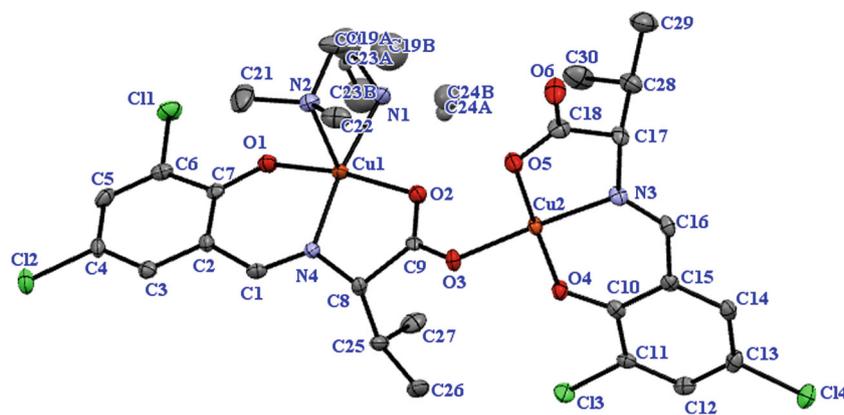


**Figure 1.** An ORTEP diagram of the complex **1** with the numbering scheme. (Hydrogen atoms are omitted for clarity).

geometry between spy and tbp.<sup>25</sup> In case of five coordinated metal complexes, angular structural parameter (index of trigonality)  $\tau$  { $\tau = (\beta - \alpha)/60$ , where  $\alpha$  and  $\beta$  are the two largest coordination angles}, helps us for distinguishing the geometry between tbp and spy.<sup>26</sup> In general,  $\tau = 0$  for an ideal spy and  $\tau = 1$  for ideal tbp geometry. Taking the angles N(2)-Cu(1)-N(1) as  $\beta$  {173.32°} and O(1)-Cu(1)-O(3) as  $\alpha$  {171.85°},  $\tau$  is calculated as 0.0245. This indicates that the geometry of the complex **1** is close to the square pyramidal with a slight distortion.

In the square pyramidal geometry of complex **1**, basal plane is occupied by imine nitrogen atom N(1), phenolate oxygen atom O(1), one of the oxygen atom O(3) of carboxylate group of Schiff base ligand and one of the nitrogen atom N(2) of tmen. The axial site is occupied by another nitrogen atom N(3) of tmen. The methylene carbon atoms of tmen namely C(15) and C(16) as well as N-methyl carbon atoms C(13) and C(14) exhibit disorder. The Cu(1)-N(1), Cu(1)-N(2), Cu(1)-O(1), Cu(1)-O(3) distances are 1.941(3) Å, 2.065(3) Å, 1.907(3) Å and 1.9499(3) Å, respectively. The basal bond angles N(1)-Cu(1)-O(1), N(2)-Cu(1)-O(1), N(2)-Cu(1)-O(3) and O(3)-Cu(1)-N(1) are 92.80(12)°, 91.29(12)°, 91.9(13)° and 83.33(11)°, respectively, and the Cu(1)-N(3) apical bond length is 2.415(4) Å and this is longer than the equatorial bonds as expected.<sup>27</sup> Further, the sum of the angles around Cu(1) is found to be 359.82°, which confirms that the central metal, Cu(II) ion lies in the plane.

**3.1b Structure description of complex 2:** An ORTEP view of the complex **2** along with the atom numbering scheme is shown in figure 2.



**Figure 2.** An ORTEP diagram of complex **2** with the numbering scheme. (Hydrogen atoms are omitted for clarity).

The coordination environment around Cu(1) is  $N_3O_2$ . The coordination of Cu(1) is constituted by the phenolate oxygen O(1), imine nitrogen N(4), oxygen atom O(2) of the carboxylate group of Schiff base ligand and the two nitrogen atoms N(1) and N(2) of tmen. By taking N(1)-Cu(1)-N(4) as  $\beta$  {171.6°} and O(1)-Cu(1)-O(2) as  $\alpha$  {169.8°}, the value of  $\tau$  is calculated as 0.03. This  $\tau$  value is nearly same as that of complex **1**. Hence the coordination geometry around Cu(1) can be described as very close to square pyramid. The basal plane of the square pyramid is coordinated by the imine nitrogen N(4) of the Schiff base ligand, phenolate oxygen atom O(1), oxygen atom O(2) of the carboxylate group and one of the nitrogen atom N(1) of the tmen, whereas the apical position is occupied by the nitrogen atom N(2) of tmen. The carboxylate group belonging to the Schiff base ligand around Cu(1) acts a tetradentate ligand, by bridging Cu(1) and Cu(2). The apical bond length 2.323 Å of Cu(1)-N(2) is longer than the basal distance 2.326 Å of Cu(1)-N(1).<sup>23</sup> The sum of the bond angles of the basal plane N(4)-Cu(1)-O(1) {92.3°}, O(1)-Cu(1)-N(1) {92.2°}, N(1)-Cu(1)-O(2) {91.9°} and O(2)-Cu(1)-N(4) {82.5°} is 358.9°, which further confirms the slight deviation of Cu(1) from the basal plane. The N-methyl carbons C(23) and C(24) as well as the methylene carbon C(19) exhibits disorder.

The coordination environment around Cu(2) is  $NO_3$ , in which the Cu(2) atom is coordinated to the Schiff base ligand via the imine nitrogen N(3), phenolate oxygen O(4) and the oxygen atom O(5) of the carboxylate group. The fourth bond is coordinated with the oxygen atom O(3) of the bridging carboxylate group from another Schiff base ligand. The sum of the bond angles O(3)-Cu(2)-O(5) {92.9°}, O(5)-Cu(2)-N(3) {84.7°}, N(3)-Cu(2)-O(4) {93.3°} and O(4)-Cu(2)-O(3) {88.6°} around Cu(2) is 359.5°, which confirms the presence of Cu(2) ion in the basal plane.

While comparing the bond distances between the metal centres with the similar group of atoms of Schiff base ligand, like phenolate oxygen Cu(1)-O(1) is 1.928 and Cu(2)-O(4) is 1.903 Å, imine nitrogen atom Cu(1)-N(4) is 1.937 and Cu(2)-N(3) is 1.922 Å and oxygen atom of the carboxylate group Cu(1)-O(2) is 1.999 and Cu(2)-O(5) is 1.923 Å, it is interesting to note that bond distances are shorter around Cu(2) when compared to that of Cu(1).

Similarly, while comparing the bond angles between the similar groups O(1)-Cu(1)-N(4) {92.3°} and O(4)-Cu(2)-N(3) {93.3°}, N(4)-Cu(1)-O(2) {82.5°} and N(3)-Cu(2)-O(5) {84.7°} it is to be noted that the bond angles around Cu(2) is greater than that of Cu(1), which may be due to the coordination environment around the central metal ion. The bond length of imine group C(1)-N(4), attached with the five coordinated Cu(II) ion is 1.285 Å and this is found to be slightly greater than the other imine group C(16)-N(3), with the bond length 1.280 Å, attached with the four coordinated Cu(II) ion. The bridging of two copper(II) ions via carboxylate group of Schiff base ligand leads to a dinuclear moiety with *syn-anti* conformation. The separation between Cu(1)...Cu(2) is 5.06 Å.

The sum of the bond angles and the  $\tau$  value around penta coordinated Cu(II) ions in both the complexes were found to be nearly same. As per the literature,<sup>28</sup> the coordination mode of NN-donor chelating diamine displays axial-equatorial mode of bonding with the Cu(II) atom with square pyramidal geometry.

### 3.2 FTIR spectroscopy

In the FTIR spectra, the band observed at 1637 and 1641  $\text{cm}^{-1}$  of the complexes **1** and **2** (figures S1 and S2, Supplementary Information), respectively, correspond to the coordinated imine group. The phenolic  $\nu(\text{C}-\text{O})$  band for the complexes **1** and **2** appeared at 1193 and

1163  $\text{cm}^{-1}$ , respectively. The carboxylate group can coordinate in different ways via monodentate or bidentate to one metal ion or it can act as a bridging ligand between two metal ions. The coordination mode of the carboxylate group can be determined by FTIR spectroscopy. For bidentate chelating or bridging mode the separation between asymmetric and symmetric stretching ( $\Delta\nu = \nu_{\text{as}} - \nu_{\text{s}}$ ) frequencies of the carboxylate group is significantly less compared to that of the free carboxylate anion value, while the separation is more, compared to the free anionic value in case of unidentate mode.<sup>29</sup>

In the present case, for complex **1** ( $\nu_{\text{as}} = 1533 \text{ cm}^{-1}$ ;  $\nu_{\text{s}} = 1340 \text{ cm}^{-1}$ ), the  $\Delta\nu$  value is  $193 \text{ cm}^{-1}$  which is higher than that of free carboxylate anion ( $145 \text{ cm}^{-1}$ ). This indicates a monodentate coordination of the carboxylate group of Schiff base ligand with copper(II) ion of complex **1**. In contrast, for complex **2**,  $\Delta\nu$  value is  $142 \text{ cm}^{-1}$  ( $\nu_{\text{as}} = 1517 \text{ cm}^{-1}$ ;  $\nu_{\text{s}} = 1375 \text{ cm}^{-1}$ ). This is in accordance with the single crystal XRD, and hence substantiate the bridging coordination mode of the carboxylate group<sup>30</sup> between two copper(II) ions of complex **2**. Further, the band observed for the complexes **1** and **2** at  $545$  and  $574 \text{ cm}^{-1}$ , respectively, could be ascribed to  $\nu(\text{Cu-N})$  coordination. While the band observed at  $487$  and  $497 \text{ cm}^{-1}$ , for the complexes **1** and **2** respectively, are associated to  $\nu(\text{Cu-O})$  bonding.<sup>31</sup>

### 3.3 Electron Spin Resonance spectra

To analyse the metal environment like geometry and the degree of covalency of metal-ligand bonds, ESR spectra of the complexes **1** and **2** (figures S3 and S4, in Supplementary Information) were recorded in polycrystalline state under liquid nitrogen temperature. For complex **1** ( $g_{\parallel} = 2.05$ ;  $g_{\perp} = 2.02$ ), the  $g$  values indicate the axial symmetry and the relation  $g_{\parallel} > g_{\perp} > 2.0023$  holds for the components of the  $g$ -tensor. The square pyramidal configuration with  $C_{4v}$  symmetry point group is consistent with the parameters of the ESR spectra obtained, which satisfy the above mentioned relation.<sup>32</sup> The geometric parameter  $G$ ,  $[(g_{\parallel} - 2.0023) / (g_{\perp} - 2.0023)]$ , is a measure of the exchange interaction between copper centres in the polycrystalline compound. It is perceived that, the exchange interactions in the solid complex is negligible if  $G > 4$ , while considerable exchange interaction occurs if  $G < 4$ . For complex **1**, the  $G$  value is found to be 1.9, which is consistent with a  $dx^2-y^2$  ground state and hence prove the presence of considerable exchange interaction in the solid state.<sup>33</sup> The ESR spectrum of the complex **2** displayed an isotropic spectrum with the  $g_{\text{iso}}$  value of 2.02, indicating an axial symmetry with all the principal axes aligned parallel. Such

a spectrum is expected in complexes with elongated tetragonal-octahedral, square planar, or square based pyramidal stereo chemistry.<sup>34</sup>

### 3.4 UV-Visible and CD spectra

The electronic property and the structure of complexes **1** and **2** in solution state were compared by UV-Visible and CD spectroscopy (figure 3) recorded in ethanol. Both these spectroscopic techniques have been used to characterize the inorganic complexes in solution state.<sup>35,36</sup>

In the electronic absorption spectrum (figures S5 and S6, in Supplementary Information), the complexes **1** and **2** exhibited an intense band at  $225 \text{ nm}$  and  $240 \text{ nm}$ , respectively, accompanied by a shoulder band around  $270 \text{ nm}$  and  $280 \text{ nm}$ , respectively. These bands are associated with  $\pi \rightarrow \pi^*$  of benzene ring/intra ligand charge transfer (ILCT) transitions. Complexes **1** and **2** displayed an absorption band at  $375 \text{ nm}$  and  $390 \text{ nm}$ , respectively, which are attributed to  $n \rightarrow \pi^*$  transition originating from electrons present on the nitrogen atom of the azomethine moiety. The difference in the absorption wavelength maxima between complexes **1** and **2** indicates that the electronic properties of these complexes are dissimilar. In UV region of the CD spectrum, complex **1** exhibited positive bands at  $223 \text{ nm}$  and  $256 \text{ nm}$  and negative bands at  $208$ ,  $240$  and  $272 \text{ nm}$ . Similar to complex **1**, positive bands at  $228$  and  $253 \text{ nm}$  and negative bands at  $211$  and  $278 \text{ nm}$  were observed for complex **2**, except a negative band at  $240 \text{ nm}$ .<sup>37</sup> Further, the relative intensities of these bands are different between complex **1** and **2**. The difference in the CD spectrum could be attributed to the difference in the structure of complexes **1** and **2**.

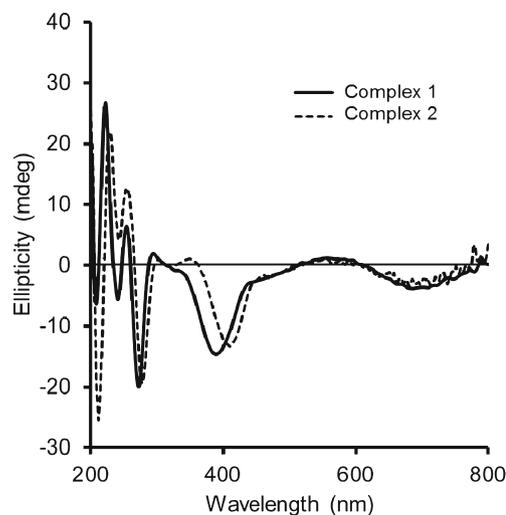


Figure 3. CD spectra of copper(II) complexes **1** and **2**.

According to the literature, the Schiff base complexes derived from salicylaldehyde, the CD bands of imine groups appeared to be negative band and flanked by a shoulder band.<sup>38</sup> Similarly, the CD spectra of the complexes **1** and **2** displayed bands at 388 nm and 412 nm, respectively, with the negative Cotton effect, associated with  $n \rightarrow \pi^*$  transition of the azomethine group. This is accompanied a band at 318 nm and 325 nm for the complexes **1** and **2**, respectively, as a shoulder band with negative Cotton effect. Again, the shift in the position of CD bands of both the complexes suggests the different geometries possessed by the complexes **1** and **2**, in accordance with the single crystal XRD. However, it should be noted that the shift could also be caused by a wavelength maximum shift, as observed in the electronic spectrum.

In UV-Visible absorption spectrum, the d-d transitions were not observed owing to lower concentration of the Cu(II) complexes while, the same can be observed at higher concentrations. In contrast, CD spectra provide valuable information regarding d-d transitions even at low concentrations of the metal complexes. In the visible region, both the complexes exhibited CD couplet, a negative band around 695 nm followed by a weak positive band around 570 nm, which is attributed to the d-d transition. Such a type of CD spectrum is due to the asymmetric coordination environment around Cu(II) ion and the same has been previously observed for the metal complexes.<sup>39</sup> The CD spectra thus suggest the asymmetric environment around Cu(II) ion in both the complexes.

### 3.5 *In vitro* antidiabetic activity

The results of glucose inhibitory assay are given in tables S3 and S4, in Supplementary information. The percentage inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase exhibited by the complexes are depicted in table S5, in Supplementary information. IC<sub>50</sub> values of complexes **1** and **2** are compared with standard drug acarbose and listed in table 1.

**Table 1.** IC<sub>50</sub> values of standard drug acarbose and the complexes.

Sample	IC <sub>50</sub> ( $\mu\text{g/mL}$ )	
	$\alpha$ -amylase	$\alpha$ -glucosidase
Acarbose	48.33 $\pm$ 0.73	42.19 $\pm$ 0.09*
Complex <b>1</b>	941.20 $\pm$ 0.09	919.02 $\pm$ 0.22
Complex <b>2</b>	389.01 $\pm$ 0.40	350.02 $\pm$ 0.24*

Values are mean SEM for group of three observations,  $p^* < 0.05$ .

Many researchers have focused on *in vivo* antidiabetic activities of copper complexes.<sup>19,40</sup> Walter *et al.*,<sup>41</sup> hypothesized that copper metabolism encounters the diabetic pathological conditions. Copper can increase the tolerance of pancreatic  $\beta$ -cells against oxidative stress, which is one of the causative agents of diabetes.<sup>42</sup> Intramuscular injection of copper(II) acetate imidazole complex to streptozotocin (STZ) induced rats are shown to increase in glucose tolerance and consequently decrease in blood glucose level. Abdul-Ghani *et al.*,<sup>43</sup> proved the antidiabetic effect of bis(acetato)tetrakis(imidazole) copper(II) in STZ induced diabetic rats. Yasumatsu *et al.*,<sup>44</sup> stated that single intraperitoneal injection of copper(II) picolinate to diabetic mice shown higher hypoglycemic effect. Moreover, Barthel *et al.*,<sup>45</sup> proved that, the copper chelating agent tetrathiomolybdate decreased serum copper ions and free radicals which ameliorates glucose and lipid metabolism in diabetic db/db mice model. The mechanism of action by copper has been suggested, that copper treats hyperglycemia by activating the phosphoinositide 3'kinase (PI3-K/Akt) pathway leading to GLUT 4 translocation<sup>46</sup> in some studies. Hence, the above studies indicate that Cu(II) complexes exhibit good antidiabetic activity.

In the current study, the inhibitory effect of copper(II) complexes **1** and **2** on carbohydrate hydrolysing enzymes  $\alpha$ -amylase and  $\alpha$ -glucosidase were investigated. These enzyme inhibitors antagonize the activity of these enzymes and delaying the digestion of carbohydrate which prevents the sudden rise in blood glucose level, especially after meal.<sup>47</sup> Therefore, inhibition of these two enzymes is an attractive approach for the management of diabetes. In the present study, complex **2** inhibits  $\alpha$ -amylase 50.18 $\pm$ 0.39% and acarbose inhibits 84.80 $\pm$ 0.03%. Complex **2** inhibits glucosidase 54.2 $\pm$ 0.05% and complex **1** inhibits 20.52 $\pm$ 0.40%; these are compared with standard drug acarbose which inhibits 85.80 $\pm$ 0.03%. Hence, complex **2** is considered as moderate inhibitor when compared with complex **1**. Complex **1** was least potent inhibitor for those enzymes. Relative movement of the complex **1** was 82.35 $\pm$ 0.91% and complex **2** was 49.97 $\pm$ 0.91%. Complex **2** effectively retards the movement of glucose through the biomembrane after three hours. This suggested that the complex **2** can delay absorption compared to complex **1**. The IC<sub>50</sub> values of the copper(II) complexes **1** and **2** were much higher when compared with standard drug acarbose. The enhanced activity of complex **2** may be due to the electronegative chloro substituents present in the Schiff base ligand. Hence, some substituent changes in these complexes may help to exhibit potential inhibition on these enzymes and less diffusion through

intestinal membrane. However, further *in vivo* studies are essential to prove their antidiabetic activity and the mechanism.

#### 4. Conclusions

In summary, synthesis and characterization of two new copper(II) complexes derived from tridentate ONO donor Schiff bases N-(salicylidene)-L-valine and N-(3,5-dichlorosalicylidene)-L-valine with tmen (N,N,N',N'-tetramethylethylene-1,2-diamine) are described in this article. Complex **1** adopts a distorted square pyramidal geometry while complex **2** adopts a *syn-anti* mode of carboxylate bridged dinuclear structure, as revealed by single crystal XRD. The IC<sub>50</sub> values of *in vitro* antidiabetic studies of both the complexes were much higher when compared with standard drug such as acarbose. Since both the complexes showed significant inhibiting activities, the current results provide a lead for the *in vivo* studies to establish the possibility of these complexes as antidiabetic agent.

#### Supplementary Information (SI)

CCDC 933779 (for complex **1**) and 919273 (for complex **2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at [www.ccdc.cam.ac.uk/conts/retrieving.html](http://www.ccdc.cam.ac.uk/conts/retrieving.html). Crystal data and structure refinement for the copper(II) complexes **1** and **2** (table S1), Selected bond lengths (Å) and angles (deg) for complexes **1** and **2** (table S2), Diffusion of glucose through dialysis membrane to the external solution concentration of glucose (mg/dL) (table S3), Relative movement of glucose through dialysis membrane over 180 minutes (table S4), Inhibitory activity of complexes at varying concentrations on  $\alpha$ -amylase and  $\alpha$ -glucosidase compared with standard drug acarbose (table S5), FTIR (figures S1, S2), ESR (figures S3, S4) and electronic absorption spectra (figures S5, S6) of the complexes **1** and **2** are given in Supplementary Information, available at [www.ias.ac.in/chemsci](http://www.ias.ac.in/chemsci).

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