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Synthesis, characterization and biological studies of a novel Cu(II) Schiff base complex

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Abstract

A Cu(II) complex was obtained from the reaction of 4-(diethylamino)-3-quinolin-3-ylimino-methyl-2-phenol with [CuCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2}]. The ligand coordinated to the metal ion in a monobasic bidentate fashion. The molecular structure of the complex has been confirmed by single crystal X-ray diffraction. Both the ligand and complex were characterized by various spectroscopic techniques. Using the UV-visible and fluorescence spectroscopic studies, the binding interactions of the compounds with CT-DNA and bovine serum albumin protein were evaluated. The results indicated that the compounds are efficient DNA/protein binders. The cytotoxicity of the ligand and complex against A549 (lung cancer) and MCF7 (breast cancer) cell lines was also investigated \textit{in vitro} conditions using the MTT assay method which showed significant anticancer activity.

Keywords: schiff base ligand, copper(II) complex, DNA/protein binding, cytotoxicity.

Introduction

Most of the drugs that are used at present for cancer treatment are cytotoxic (cell-killing), affecting in one way or the other with the functioning of the DNA in cells. However, the biggest challenge in the development of new anticancer drugs is to find chemotherapeutic agents not only with high efficacy but also with low toxicity. It has been observed that though cisplatin has been used most successfully in the treatment of various types of cancers, it has been associated with side effects in addition to inherited or acquired resistance [1,2]. Hence, chemists all over the world have been forced to look for an alternative approach in finding human friendly metal complexes to enhance the pharmacological properties. From among the various metal complexes studied so far, copper ion based complexes have been proved to be a promising alternative to platinum drugs [3]. Copper-based antineoplastic agents have shown remarkable results not only to test the altered metabolism of cancer cells but also to differentiate between cancer and normal
cells [4]. In this connection, several copper-based synthetic nucleases have been reported [5-7] and a few of them have been found to cleave DNA hydrolytically [8,9]. During the last several years, there has been a consistent and continuous effort in finding out the mode and extent of binding of metal complexes to DNA and protein and also to obtain vital information on understanding the cleavage properties of metal complexes for their biological applications. Numerous inorganic chemists have chosen copper for its bio-essential activity and oxidative nature to establish its complexes for medical applications [10–13]. In this area, copper complexes containing heterocyclic bases as ligands have been extensively explored in view of their strong interactions with DNA and of their cytotoxic activity [14–16]. In addition to this, copper complexes with their biologically accessible redox potentials have shown high nucleobase affinity with DNA [17-21] besides exhibiting an essential alternative to platinum drugs [22-28]. From the point of ligand used to prepare useful metal complexes, the combination of nitrogen containing heterocyclic ring and azomethine moiety might prove to be useful due to their potential biological and catalytic activities. Further, quinolines as well as their derivatives are also a class of ligands exhibiting a wide range of biological applications. In fact, some quinoline based Schiff base ligands and their Cu(II) complexes have been reported to show interesting pharmacological properties [29-34]. Our perusal on the literature has indicated that no attention has been paid to explore the biological properties of 4-(diethylamino)-3-quinolin-3-ylimino-methyl-2-phenol and its Cu(II) complexes. Hence, we carried out the reaction of 4-(diethylamino)-3-quinolin-3-ylimino-methyl-2-phenol with [CuCl₂(PPh₃)₂] and report the biological studies done on the ligand and that of the new complex.

**Results and discussion**

**Synthesis**

The synthetic route to the ligand and its Cu(II) complex are shown in **Scheme 1** and they have been characterized by various spectroscopic techniques as discussed below. The ligand and complex are air stable and soluble in chloroform, dichloromethane, methanol, ethanol, dimethylformamide and dimethylsulfoxide.
**Scheme 1.** Synthetic route of the ligand and its Cu(II) complex.

**Spectroscopy**

In the electronic spectra of ligand, three bands were observed in the region 282-402 nm, which are assigned to intra ligand transitions. Spectra of the complex exhibited two bands, the band observed at 254 nm was assigned to intra ligand transition and the band at 383 nm corresponds to ligand to metal charge transfer transition [35-37]. The IR spectrum, a band observed at 1628 cm\(^{-1}\) due to the C=N stretching of the ligand has been found shifted to 1606 cm\(^{-1}\) in the spectra of the complex, indicating coordination of the azomethine nitrogen atom to the Cu(II) ion. The band at 3432 cm\(^{-1}\) due to the –OH group in the free ligand disappeared completely in the spectra of the complex due to coordination of phenolic oxygen after deprotonation [38,39]. The \(^1\)H-NMR spectra of the ligand (Figure S1), showed a sharp singlet at \(\delta\) 8.93 ppm corresponding to the presence of a hydroxy group in the free ligand. A doublet at \(\delta\) 8.42-8.43 ppm corresponds to the presence of the CH=N azomethine proton [40]. While a quartet observed around at \(\delta\) 2.49-2.53 ppm corresponds to the CH\(_2\)N group of protons in the ligand, a triplet observed at \(\delta\) 1.11-1.14 ppm has been assigned to the terminal methylene group of protons [39]. The aromatic protons resonances of the ligand were observed in the range at \(\delta\) 6.11-7.99 ppm. \(^{13}\)C NMR spectra of the ligand showed the chemical shift values of each carbon in the expected region (Figure S2). The signal observed at \(\delta\) 152.40 ppm has been assigned to the azomethine (CH=N) carbon [41], with the aromatic carbons resonances in the region of 168.32-109.40 ppm [42]. The -CH\(_2\) carbon
signal appeared at δ 49.21 ppm and the -CH₃ carbon was observed at 17.78 ppm in the ligand respectively [43,44]. The +2 oxidation state of the copper ion in the complex was confirmed by X-band EPR measured at room temperature. The copper complex showed well resolved isotropic resonance typical of square planar Cu(II) system (Figure S3). From the g value of 2.01, it is proposed that the unpaired electron of Cu(II) ion is present in the dₓ₂−ᵧ₂orbital [45].

**X-ray crystallography**

The complex crystallized in the triclinic space group P-1 with half a molecule in the asymmetric unit (Z′=0.5). Two ligands coordinated to the copper ion in a monobasic bidentate fashion through the imine nitrogen (N1) and phenolic oxygen (O1) forming a six-member chelate rings. The Cu(II) ion showed approximately square planar geometry with the two unique O-Cu-N angles of 92.03(4)° and 87.97(4)°, the rms deviation of the Cu from the ONON plane was necessarily 0 as a result of the Cu being sat on an inversion center. Surprisingly, the bidentate chelating ligand replaced all the triphenylphospine ligands and chloride ions from the Cu(II) precursor (Figure 1). Crystal data and refinement details for the complex are presented in (Table 1). Important bond Length (Å) and bond angle (°) of the complex given in Table S1 (supporting information).

![Crystal structure](image)

**Figure 1.** Crystal structure of the complex with atomic numbering scheme shown. Hydrogen atoms are omitted for clarity and anisotropic displacement parameters are depicted at the 50 % probability level. Symmetry code ¹ = -x, 1-y, -z.
Table 1. Crystal data and structure refinement for complex

<table>
<thead>
<tr>
<th>Identification code</th>
<th>Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>C_{40}H_{40}CuN_{6}O_{2}</td>
</tr>
<tr>
<td>Formula weight</td>
<td>700.32</td>
</tr>
<tr>
<td>Temperature/K</td>
<td>100(2)</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>$P \overline{1}$</td>
</tr>
<tr>
<td>$a$/Å</td>
<td>8.8927(4)</td>
</tr>
<tr>
<td>$b$/Å</td>
<td>9.2553(4)</td>
</tr>
<tr>
<td>$c$/Å</td>
<td>12.1514(5)</td>
</tr>
<tr>
<td>$\alpha$/°</td>
<td>106.708(2)</td>
</tr>
<tr>
<td>$\beta$/°</td>
<td>100.885(4)</td>
</tr>
<tr>
<td>$\gamma$/°</td>
<td>114.778(2)</td>
</tr>
<tr>
<td>Volume/Å^3</td>
<td>846.67(7)</td>
</tr>
<tr>
<td>Z</td>
<td>1</td>
</tr>
<tr>
<td>$\rho_{\text{calc}}$/g/cm^3</td>
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<tr>
<td>$\mu$/mm^−1</td>
<td>0.691</td>
</tr>
<tr>
<td>F(000)</td>
<td>367.0</td>
</tr>
<tr>
<td>Crystal size/mm^3</td>
<td>0.43 × 0.32 × 0.20</td>
</tr>
<tr>
<td>Radiation</td>
<td>MoKα (λ = 0.71073)</td>
</tr>
<tr>
<td>2θ range for data collection/°</td>
<td>3.594 to 55.882</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-11 ≤ h ≤ 11, -12 ≤ k ≤ 12, -15 ≤ l ≤ 15</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>15639</td>
</tr>
<tr>
<td>R_{int} / R_{sigma}</td>
<td>0.0199 / 0.0179</td>
</tr>
<tr>
<td>Data/restraints/parameters</td>
<td>4054/0/225</td>
</tr>
<tr>
<td>Goodness-of-fit on F^2</td>
<td>1.063</td>
</tr>
<tr>
<td>Final R indexes [I&gt;=2σ (I)]</td>
<td>R_1 = 0.0259, wR_2 = 0.0674</td>
</tr>
<tr>
<td>Final R indexes [all data]</td>
<td>R_1 = 0.0272, wR_2 = 0.0682</td>
</tr>
<tr>
<td>Largest diff. peak/hole / e Å⁻³</td>
<td>0.39/-0.31</td>
</tr>
</tbody>
</table>

DNA binding studies

In order for any compound to be tested for treating multiple pathologies including cancer, it is necessary to investigate the binding of such compound with DNA [46,47]. One of the easiest ways is to observe the changes in the electronic absorption spectra of the compounds as a function of added DNA. Hence, we carried out the interaction of our compounds (10 µM) with
DNA (0-100 µM) and the spectral changes observed are shown in (Figure 2). It can be seen from the figure that as and when the concentration of DNA is increased, a hyperchromism with a blue shift of 4 nm was observed for the ligand. But, for the complex, a hypochromism with a red shift of 3 nm was observed which indicates the existence of an intercalative mode of binding [48]. Generally, compounds binding to DNA through intercalation results in hyperchromism or hypochromism with or without a small red or blue shift, due to a strong stacking interaction between the planar aromatic chromophore of the compound and the base pairs of DNA [49,50]. Further, to get an idea on the binding strength of the compounds, the intrinsic binding constants ($K_b$) of them with CT-DNA have to be determined from the following equation.

$$\frac{[\text{DNA}]}{[\varepsilon_a-\varepsilon_f]} = \frac{[\text{DNA}]}{[\varepsilon_b-\varepsilon_f]} + \frac{1}{K_b[\varepsilon_b-\varepsilon_f]}$$

where, [DNA] is the concentration of DNA in base pairs, $\varepsilon_a$ is the extinction coefficient of the complex at a given DNA concentration, $\varepsilon_f$ is the extinction coefficient of the complex in free solution and $\varepsilon_b$ is the extinction coefficient of the complex when fully bound to DNA. Figure 3 shows the plot of $[\text{DNA}]/(\varepsilon_a-\varepsilon_f)$ versus [DNA] which gave a slope and an intercept equal to $1/[\varepsilon_a-\varepsilon_f]$ and $1/K_b[\varepsilon_b-\varepsilon_f]$. The intrinsic binding constant $K_b$ is the ratio of the slope to the intercept and binding constants of $0.5873 \times 10^5$ and $1.34942 \times 10^5$ M$^{-1}$ have been obtained for the ligand and the complex indicating that the complex exhibited a better binding than that of the free ligand. The higher binding affinity of the Cu(II) complex may be due to the extension of the $\pi$ system of the intercalated ligand through coordination to the metal ion and due to a greater planar area of the complex than that of the free ligand thereby penetrating more deeply into and stacks more strongly with the base pairs of DNA.
Figure 2. Electronic titration spectra of ligand (A), complex (B). Arrow shows that the absorption intensities increase and decrease upon increasing DNA concentration.

Figure 3. Plots of [DNA]/(ε_a-ε_f) versus [DNA].

Protein binding studies
In addition to DNA binding, it is further essential to know the nature of the binding of compounds to proteins to obtain information on the relationship between structure and functions of proteins. Such information can be obtained from the changes observed in the fluorescence
spectrum of BSA (Bovine Serum Albumin) on the incremental addition of new compounds to BSA. Hence, we titrated BSA with different concentrations of the compounds (0-50 μM) and the results are shown in (Figure 4). It can be noticed that there is a significant decrease in the fluorescence intensity of BSA on the subsequent addition of the compounds (A = ligand, B = complex). The decrease in fluorescence intensity with the blue shift does indicate the interaction among the ligand and complex with BSA. Using the Stern–Volmer relationship $I_0/I = 1 + K_q [Q]$ (where $I_0$ and $I$ demonstrate the fluorescence intensities in the absence and presence of a quencher, respectively, $K_q$ is a linear Stern–Volmer quenching constant, and $[Q]$ is the quencher concentration), one can calculate the quenching constant ($K_q$) from the plot of $I_0/I$ versus $[Q]$ (Figure 5). Further, the number of binding sites ($n$) and the binding constant ($K_b$) values can also be obtained from Scatchard equation $\log[(F_0− F)/F] = \log K_b + n \log[Q]$ (where $K_b$ is the binding constant of the compounds with BSA and $n$ is the number of binding sites) when small molecules bind independently to a set of equivalent sites on a macromolecule. From the plot of $\log[(F_0− F)/F]$ versus $\log[Q]$ (Figure 6), the number of binding sites ($n$) and the binding constant ($K_b$) values have been obtained. The calculated value of $n$ is around 0.69 and 1.1 for the ligand and complex, showing the existence of a single binding site in BSA for the compounds. The values of $K_q$ and $K_b$ are shown in Table 2 which indicate that the complex interacts with BSA more strongly than the free ligand, a result which is similar to the one obtained from the CT-DNA absorbance titration [51].

**Table 2.** Quenching constant ($K_q$), binding constant ($K_b$), and number of binding sites ($n$) values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_q$ (M$^{-1}$)</th>
<th>$K_b$ (M$^{-1}$)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>$1.17 \times 10^2$</td>
<td>$6.41 \times 10^3$</td>
<td>0.69</td>
</tr>
<tr>
<td>Complex</td>
<td>$2.06 \times 10^4$</td>
<td>$8.13 \times 10^3$</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Figure 4. Fluorescence quenching titration of BSA with the compounds.

Figure 5. Stern–Volmer plot of the compounds with BSA.
In these types of studies, it is also important to determine the mechanism of quenching since there are two type of quenching namely, dynamic and static. Fluorophore-quencher complex formation in the ground state refers to static quenching while such a formation in the excited state corresponds to dynamic quenching. UV-Vis absorption spectroscopy can be employed to determine the type of quenching and hence, we measured the UV-Vis spectra of BSA in the absence and presence of the test compounds and the spectra recorded are shown in (Figure 7). The addition of the Cu complex to a fixed concentration of BSA led to a gradual increase in the intensity of BSA absorption at the same wavelength due to the interaction between the complex and protein, which is ascribed to the static quenching [52].
Synchronous fluorescence spectra

Tyrosine, tryptophan and phenylalanine residues are responsible for the fluorescence properties of BSA and one can find out the presence of any of these residues by measuring the synchronous fluorescence spectra of BSA with the compounds under study [53]. Hence, we measured the synchronous fluorescence spectra of BSA in the presence and absence of increasing concentrations (0-50 μM) of compounds at different Δλ. It is well known that at Δλ = 15 nm is characteristic of tyrosine residue, while the corresponding spectrum at Δλ = 60 nm is characteristic of tryptophan. The synchronous fluorescence spectra of BSA with various concentrations of test compounds were recorded. On the adding of the compounds to BSA, the fluorescence intensity at 342 nm decreased both for the ligand and the complex (Figure 8). The synchronous fluorescence spectra results clearly showed that the fluorescence intensity of both tryptophan and tyrosine micro-regions was affected with increasing concentration of the compounds [54-56].
Figure 8. Synchronous fluorescencetitrination of BSA with the compounds at $\Delta \lambda = 60$ nm (A= ligand, B = complex).

Cytotoxicity

The positive results obtained from the DNA and protein binding studies prompted us further to explore their in vitro cytotoxicity on cancer cell lines and we carried out the studies against A549 (lung cancer) and MCF7 (breast cancer) cell lines. The cell death inducing ability of the compounds was monitored by using the AO/EB staining assay and DAPI fluorescence study. The inhibition at 24 h of cancer cell growth at 50 % level and IC$_{50}$ values of the ligand and the complex are shown in (Table 3) and (Figure 9). It is important to mention that the copper(II) complex presented herein showed better growth inhibitory effect than the previously reported copper complexes [57-60]. The cytotoxic properties of this new Cu(II) complex can be attributed to the extended planar structure induced by the chelation of the quinoline ligand to the copper ion in the complex. The ligand also exhibited a moderate cytotoxic effect on A549 and MCF7 cancer cell lines compared to doxorubicin and cisplatin, with the copper(II) complex showed better activity against MCF7 cell line.
Table 3. The IC$_{50}$ values of the Compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>A549</th>
<th>MCF7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>34± 1.8</td>
<td>27± 1.7</td>
</tr>
<tr>
<td>Complex</td>
<td>30± 1.5</td>
<td>24± 1.5</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>15± 1.2</td>
<td>16± 1.3</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>25 ± 2.1</td>
<td>18.7 ± 0.1</td>
</tr>
</tbody>
</table>

**Acridine orange /Ethidium bromide (AO/EtBr) staining Method**

To establish the potential of any compound for a therapeutic use, it is essential to look at the apoptotic activity of the compound through apoptotic staining fluorescence microscopic analysis. Hence, the fluorescence microscopic images of A549-A and MCF7-B cancer cells in the absence (control) and in the presence of the free ligand and the new Cu complex have been recorded and the results are shown in (Figure 9). From the figure, it is clear that the untreated A549 and MCF7 cancer cells (control (a), (b)) did not show any significant adverse effect compared to that treated with the compounds (treated (c), (d)). Besides, it can also be seen that on the addition of the test compounds to the cancer cells, the green colour of cells are converted into orange/red colour cells which is due to induced apoptosis and the nuclear condensation effect on the cells. The results do indicate that the compounds have the ability to significantly induce the apoptosis in selected cancer cells.
Figure 9. AO/EB stained A549 (A) and MCF7 (B) (a, b control and c, d, are treated with the ligand and complex) cells after 24 h incubation. The yellow or red color cells indicate early apoptotic cells.

**DAPI staining method**

Since it is necessary to confirm the nuclear condensation and fragmentation of the test compounds, fluorescence microscopy images of lung and breast cancer cells were taken after staining with DAPI for 24 h in the absence and presence of compounds and the results are shown in (Figure 10). It can be seen from the figure that the untreated cells (a, b) didn’t show any significant changes whereas the ones treated with the compounds (c, d) showed bright patches, which suggest the presence of condensed chromatin and nuclear fragmentations in the cancer cells. Thus, from the results of MTT assay and fluorescence microscopy analysis, we feel that the new complex has the potential to be tried as a potent therapeutic agent.
Figure 10. DAPI images A549 (A) and MCF7 (B) (a, b control and c, d, are treated with the ligandand complex). The blue patches show nuclear fragmentations in the cancer cells.

Conclusion

A new Cu(II) complex synthesized has been fully characterized by using analytical and various spectral studies including the structure by X-ray crystallographic techniques. The ligand coordinated to the metal ion in a monobasic bidentate fashion. Binding study of both the ligand and complex with CT-DNA has been carried out which showed that they interact through intercalation mode. From the UV-visible and fluorescence spectroscopic studies, the binding interactions of the compounds with CT-DNA and bovine serum albumin protein were evaluated. Furthermore, the compounds were tested against A549 (lung cancer) and MCF7 (breast cancer) cell lines and the results indicated significant cytotoxicity as evidenced by the IC\textsubscript{50} values compared to the doxorubicin and cisplatin. The morphological changes examined by staining method suggested that the cell death mechanism was through apoptosis. The higher binding affinity and cytotoxic activity of the Cu(II) complex may be due to the extension of the π system of the intercalated ligand.

EXPERIMENTAL SECTION

Materials and methods

All the chemicals were purchased from sigma-Aldrich and were used as received. In the case of solvents, standard procedures were followed to purify them [61]. The metal complex, [CuCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2}] and ligand were prepared following the procedures reported in the literature.
Infrared spectra of the ligand and the metal complex were recorded as KBr discs using a Jasco FT-IR spectrophotometer. The melting points were recorded with a Lab India melting point apparatus. Elemental analyses were performed on a Vario EL III Elementaranalyser instrument. The electronic spectra of the compounds were recorded using a Jasco V-630 spectrophotometer. Emission spectra were measured using a Jasco FP 6600 spectrofluorometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AMX 500 NMR spectrometer. The X-band EPR spectrum of the complex was recorded at room temperature. DNA binding, protein binding [33,34] and cytotoxicity [64] experiments were performed using the reported procedures.

**Synthesis of ligand**

4-(diethylamino)-3-quinolin-3-ylimino-methyl-2-phenol was prepared as per the literature method [63] using 3-amino quinoline (1.4 g 0.01 mol) and 4-(Diethylamino)salicylaldehyde (1.9 g 0.01 mol). On cooling the of the reaction solution, a dark brown precipitate was formed which was filtered off. It was then washed with ethanol and dried under vacuum. Yield: 76 %. M.p.: 186 °C. Elemental analysis calculated for C$_{20}$H$_{21}$N$_3$O (%): C, 75.21; H, 6.63; N, 13.16. Found (%): C, 75.18; H, 6.57; N, 13.11. FT-IR (cm$^{-1}$) with KBr disk: 3432 ($\nu$OH), 1628 ($\nu$C=H=N). UV-Visible (DMSO), $\lambda_{max}$ (nm) [$\varepsilon_{max}$ (dm$^3$ mol$^{-1}$ cm$^{-1}$)]: 282, 331, 402 (Intra-ligand transition). $^1$H NMR (DMSO-$d_6$, $\delta$ ppm, J Hz): 8.93 (s, 1H, -OH), 8.42 (d, 1H, C=N, J=4), 7.1-7.99 (m, 6H, (Ar-H)), 6.35-6.37 (d, 1H, J=8, (Ar-H)), 6.11-6.12 (d, 1H, J=4, (Ar-H)), 6.10 (s, 1H, (Ar-H)), 2.49-2.53 (q, 4H, -CH$_2$N), 1.11-1.14 (t, 6H, -CH$_3$). $^{13}$C (DMSO-$d_6$, $\delta$ ppm): 168.32 (C17), 152.40 (C11), 152.18 (C10), 150.92 (C3), 147.34 (C2), 139.71 (C15), 133.49 (C13), 133.18 (C9), 132.32 (C8), 131.63 (C6), 130.74 (C7), 129.06 (C5), 128.57 (C4), 116.60 (C14), 114.01 (C16), 109.40 (C12), 49.21 (-CH$_2$, C), 17.18 (-CH$_3$, C).

**Synthesis of the Cu(II)complex**

A methanolic solution (10 cm$^3$) of 4-(diethylamino)-3-quinolin-3-ylimino-methyl-2-phenol (0.048 g 0.1517 mmol) was slowly added to [CuCl$_2$(PPh$_3$)$_2$] (0.100 g 0.1517 mmol) dissolved in chloroform (20 cm$^3$) and the mixture was heated under reflux for 5 hours. Upon cooling, the resulting solution afforded a reddish brown colored precipitate. It was filtered, washed with petroleum ether (60-80 °C) and dried in vacuum and recrystallized from methanol/DMF. The reddish brown crystals obtained were suitable for X-ray diffraction. Yield: 47 %. M.p.:198 °C. Elemental analysis calculated for C$_{40}$H$_{40}$N$_6$O$_2$Cu (%): C, 68.60; H, 5.76; N, 12.00. Found (%): C, 68.54; H, 5.69; N, 11.94. FT-IR (cm$^{-1}$) with KBr disk: 1606 ($\nu$C=H=N). UV-visible (DMSO),
λ_{max}(nm) \ [\varepsilon_{max} (\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1})]: 254 \text{ (Intra-ligand charge transfer)}; 383 \text{ (Ligand to metal charge Transfer)}. EPR (X-band) at room temperature showed a spectrum with a ‘g’ value of 2.01.

**Crystallography**

X-ray diffraction experiments of the complex was carried out at 100(2) K on a Bruker APEX II diffractometer using Mo-Kα radiation (λ = 0.71073 Å). Data collections were performed using a CCD area detector. Intensities were integrated in SAINT [65] and absorption corrections were carried out based on equivalent reflections using SADABS [66]. The structure was solved using Superflip [67,68] and refined against $F^2$ in SHELXL [69,70] using Olex2 [71]. All of the non-hydrogen atoms were refined anisotropically while all of the hydrogen atoms were located geometrically and refined using a riding model. Crystallographic data for the complex have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 1550949. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax(+44) 1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

**References**