Chapter II

Copper(I) hydrazone complexes: Synthesis, structure, DNA binding, radical scavenging and computational studies

Abstract

Potential bidentate hydrazone ligands, HL\textsuperscript{1} (1) and HL\textsuperscript{2} (2) prepared by the condensation of benzaldehyde or furfuraldehyde with benzhydrazide upon reaction with [CuCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2}] yielded corresponding mononuclear complexes of the composition [Cu(L\textsuperscript{1})(PPh\textsubscript{3})\textsubscript{2}] (3) and [Cu(L\textsuperscript{2})(PPh\textsubscript{3})\textsubscript{2}] (4). The exact nature of coordination of the hydrazones to the metal ion and the structure of the complexes were confirmed by spectral and single crystal X-ray diffraction studies. Interestingly, the reactions of 1 and 2 with [CuCl\textsubscript{2}(PPh\textsubscript{3})\textsubscript{2}] resulted in the formation of first structurally characterized copper(I) hydrazone complexes 3 and 4 along with the previously reported complex [CuCl(PPh\textsubscript{3})\textsubscript{3}] 3\textsubscript{a} (or) 4\textsubscript{a} as a minor product in both the reactions. The metal complexes 3 and 4 showed significant binding towards calf thymus DNA (CT DNA) via groove binding mode with binding constants in the magnitude 10\textsuperscript{4}-10\textsuperscript{5} M\textsuperscript{-1}. In addition, the antioxidant activities of the complexes were also investigated through scavenging effect on DPPH\textsuperscript{˙}, NO\textsuperscript{˙} and OH\textsuperscript{˙} radicals. The density functional theory calculations of the complex 4 also supported the structure and stability of the reduced complex.

Since the success of cisplatin and related platinum complexes as anticancer agents, developing other active transition metal complexes with better anticancer efficiency has attracted the attention of many research groups and become a central research theme in bioinorganic chemistry. In the search towards new metallic species with biological applications, copper compounds have proved to be excellent candidates.\textsuperscript{1-7} The specific roles of ligands analogous to the molecules that bind to or modulate the function of biological receptors make them good candidates for drug development. In recent years, the design and synthesis of new ligands with combined biological, structural and coordination properties have burgeoned. Interest in these studies stems not only due to the different coordination modes but also to their chemical reactivity. Hydrazine derivatives containing N-heterocycles and their complexes exhibit strong antitumor and antivirus activities.\textsuperscript{8,9} Metal complexes constitute an important subset of DNA-binding compounds, often DNA-intercalators and groove binders,\textsuperscript{10-12} the latter characterized by non-covalent sequence specific interactions.\textsuperscript{13}
The studies of copper(II) complexes have been widely explored for the versatility of their coordination geometries, exquisite colours, technical application dependent molecular structures, spectroscopic properties and their biochemical significance. Octahedral copper(II) complexes of the ligand containing mixed electron donors have been studied extensively due to their potential applications as molecular materials.\textsuperscript{14-16} In recent years considerable research efforts have been focused on synthesis and properties of copper(II) complexes of hybrid ligands because they can provide new materials with useful properties such as magnetic exchange,\textsuperscript{17} electrical conductivity,\textsuperscript{18} photoluminescence,\textsuperscript{19} nonlinear optical property\textsuperscript{20} and antimicrobial activity.\textsuperscript{21} Among various ligands, arylazo ligands containing azoimine (–N=N–C=N–) group of π-acidic N, N′-chelating systems and their coordination chemistry is presently an active area of research.\textsuperscript{22-24} The azoimine function has a specialty to stabilize lower oxidation states of metal ions and tunes the redox and spectroscopic properties of the metal centre.\textsuperscript{25,26} Due to presence of azoimine group, these compounds possess several distinctive properties including aggregation, optical data storage and tautomerisation which define as a distinct class of dyestuffs.\textsuperscript{27,28} 1,2-bis(diphenylphosphino)ethane (dppe) is one of the most versatile phosphine ligand, capable of binding to the metal ions in variety of ways: monodentate, chelating or bridging. They possess high covalency as well as ligand field effect to enforce a drastic change in magnetic and other behaviour of the resulting compounds.\textsuperscript{29}

Herein, we report the synthesis, structural investigation of hydrazone-based ligands and their new copper(I) complexes along with studies on the DNA binding and antioxidant activities. Further, a quantum chemical investigation on the complex 4 has been carried out by applying the density functional theory (DFT).

Experimental section

Materials

The following reagent grade chemicals were purchased and used without further purification. CuCl\textsubscript{2}-2H\textsubscript{2}O, triphenylphosphine, furfuraldehyde, benzaldehyde, benzhydrazide were purchased from Sigma-Aldrich chemical company and all the other
chemicals and reagents used for DNA binding and antioxidant studies were of high quality and commercially available from reputed suppliers.

**Physical measurements**

Elemental analyses (% C, H & N) were performed on a Vario EL III CHNS analyzer and all IR spectra were recorded using KBr pellets on a Nicolet Avatar instrument in the frequency range 400-4000 cm\(^{-1}\). Electronic spectra of the ligands and their complexes were recorded in DMSO-buffer on a Jasco V-630 spectrophotometer and the fluorescence spectra were measured on a Jasco FP 6600 spectrofluorometer in a DMSO-buffer medium. The \(^1\)H NMR spectra were recorded on a Bruker AMX 500 spectrometer operating at 500 MHz using tetramethylsilane as an internal standard and CDCl\(_3\) as a solvent. The cyclic voltammetric study was carried out with a CH Instruments electrochemical analyser in dichloromethane using tetrabutylammonium perchlorate (TBAP) as supporting electrolyte. A three electrode assembly comprising a glassy carbon working electrode, a platinum wire auxiliary electrode and an Ag/AgCl reference electrode was employed in this study. All solutions were purged with nitrogen gas prior to making measurements at room temperature.

The X-ray diffraction data of the complexes 3\(a\) and 4 were collected at 293 K with MoK\(\alpha\) radiation (\(\lambda = 0.71073\) Å) using a Bruker Smart APEX II CCD diffractometer equipped with graphite monochromator. The structures were solved by direct methods and refined full-matrix least squares (on F\(^2\)) SHELXS97 and SHELXL97\(^{30}\) and the graphics were produced using PLATON97.\(^{31}\) All the non-hydrogen atoms were refined anisotropically and the hydrogen atoms were positioned geometrically and refined as riding model.

**Synthesis of starting precursor complex**

The requisite precursor metal complex [CuCl\(_2\)(PPh\(_3\))\(_2\)] was prepared according to the literature method.\(^{32}\)
Preparation of dichlorobis(triphenylphosphine)copper(II), \([\text{CuCl}_2(\text{PPh}_3)_2]\)

To a warm solution of CuCl$_2$·6H$_2$O (0.8524 g; 0.005 mol) a hot solution of triphenylphosphine (2.623 g; 0.01 mol) in minimum amount of ethanol was added slowly with constant stirring to give a white precipitate. The reaction mixture was boiled for 5 minutes and kept at room temperature for 24 hours. The precipitate was filtered, washed with dry acetone and dried in a vacuum desiccator. The complex was further washed with petroleum ether to remove traces of free triphenylphosphine.

Yield: 78%. Colour: White; mp: 222 °C.

Synthesis of hydrazone ligands

The reactions involved in the synthesis of hydrazone ligands and their corresponding copper complexes are given in scheme 2.1.

\[
\text{R} \cdot \text{O} + \text{H}_2\text{N} - \text{NH} \rightarrow \text{EtOH} \text{ Reflux, 5h}
\]

\[
\text{H} \cdot \text{N} - \text{N} \cdot \text{O} \rightarrow \text{MeOH/KOH} \text{ Reflux, 8h} \rightarrow \text{[CuCl}_2(\text{PPh}_3)_2]\]

\[
\text{PPh}_3 \ \text{PPh}_3 \ \text{PPh}_3 \ \text{PPh}_3
\]

\[
(3 \text{ or } 4) \quad (3a \text{ / } 4a)
\]

where, \( \text{R} = \text{C}_6\text{H}_5 (\text{HL}_1); 3 \text{ or } \text{C}_4\text{H}_3\text{O} (\text{HL}_2); 4 \)

\[\text{Scheme 2.1 Synthesis of hydrazone ligands and their Cu(I) complexes.}\]
Synthesis of benzoic acid benzylidene-hydrazide ligand \( \text{HL}_1 \) (1)\(^{33} \)

\( \text{HL}_1 \) ligand was prepared by refluxing equimolar mixture of benzaldehyde (0.106 g; 1 mM) with benzhydrazide (0.136 g; 1 mM) respectively in 50 mL of ethanol for 5h as summarized in scheme 2.1. The reaction mixture was cooled to room temperature and then the product formed was filtered and washed several times with distilled water, then recrystallized from MeOH to afford the desired product.

Yield: 85%. Colour: White; mp: 153 °C. Anal. Found (%) for \( \text{C}_{14}\text{H}_{12}\text{N}_2\text{O} \) (Mol wt = 224.25): C, 74.08; H, 5.12; N, 12.03. Calculated (%): C, 74.98; H, 5.39; N, 12.49. Selected IR bands (\( \nu_{\text{max}} \) in cm\(^{-1} \)): 3185 (NH); 1644 (C=O); 1546 (C=N); 1070 (N–N). UV-visible (DMSO-buffer): \( \lambda_{\text{max}} \) (nm): 231 & 330 (ILCT). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \) (ppm): 9.22 (s, 1H, NH); 8.14 (s, 1H, CH=N); 7-8 (m, 10H, Ar–H).

Synthesis of benzoic acid furan-2-yl-methylene-hydrazide ligand \( \text{HL}_2 \) (2)\(^{34} \)

\( \text{HL}_2 \) ligand was prepared by refluxing equimolar mixture of furfuraldehyde (0.096 g; 1 mM) with benzhydrazide (0.136 g; 1 mM) respectively in 50 mL of ethanol for 5h as summarized in scheme 2.1. The reaction mixture was cooled to room temperature, and then the product formed was filtered and washed several times with distilled water, then recrystallized from MeOH to afford the desired product.

Yield: 85%. Colour: White; mp: 190 °C. Anal. Found (%) for \( \text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_2 \) (Mol wt = 214.22): C, 66.99; H, 4.32; N, 12.89. Calculated (%): C, 67.28; H, 4.71; N, 13.08. Selected IR bands (\( \nu_{\text{max}} \) in cm\(^{-1} \)): 3245 (NH); 1642 (C=O); 1539 (C=N); 1072 (N–N). UV-visible (DMSO-buffer): \( \lambda_{\text{max}} \) (nm): 235 & 335 (ILCT). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta \) (ppm): 9.24 (s, 1H, NH); 8.22 (s, 1H, CH=N); 7-8 (m, 10H, Ar–H).

Synthesis of metal hydrazone complexes

**Synthesis of \([\text{Cu(L}_1\text{)(PPh}_3\text{)}_2] \) (3 and 3a)**

Complex 3 was prepared by refluxing equimolar quantities of the ligand \( \text{HL}_1 \) (1) (0.224 g; 1 mM) and \([\text{CuCl}_2\text{(PPh}_3\text{)}_2]\) (0.658 g; 1 mM) in 40 mL of methanol (scheme 2.1). After 30 min, few drops of alc.KOH were added to the reaction mixture, following which it was refluxed further for 8h. After cooling the reaction mixture to room
temperature, the resulting precipitate was filtered, washed with methanol and dried in vacuo for 24 h. Purity of the product checked by TLC showed two distinct spots that were separated by column chromatography. The fractions obtained with 75:25 petroleum ether and ethylacetate mixture as the eluent yielded complex 3. Another product obtained by using 85:15 petroleum ether/ethylacetate mixture as the eluent resulted in the crystallisation of complex 3a suitable for single crystal X-ray diffraction study. The structure of 3a was shown to be identical to that of [Cu(Cl)(PPh₃)₃].

Yield: 51%. Colour: Yellow; mp: 153 °C. Anal. Found (%) for C₅₀H₄₁N₂O₂P₂Cu (Mol wt = 811.36): C, 73.88; H, 4.91; N, 3.01. Calculated (%): C, 74.02; H, 5.09; N, 3.45. Selected IR bands (ν max in cm⁻¹): 1583 & 1507 (C=N–N=C); 1355 (enolic, C–O); 1082 (N–N). UV-visible (DMSO-buffer): λ max (nm): 214 & 288 (ILCT); 371 (LMCT). ¹H NMR (500 MHz, CDCl₃): δ (ppm): 8.32 (s, 1H, CH=N); 7.31–7.85 (m, 4H, Ar–H).

**Synthesis of [Cu(L²)(PPh₃)₂] (4 and 4a)**

Complex 4 was prepared by refluxing equimolar quantities of the ligand HL² (0.213 g; 1 mM) and [CuCl₂(PPh₃)₂] (0.658 g; 1 mM) in 40 mL of methanol (scheme 2.1). After 30 min, few drops of alc.KOH were added to the reaction mixture, following which it was refluxed further for 8h. After cooling the reaction mixture to room temperature, the resulting precipitate was filtered, washed with methanol and dried in vacuo for 24 h. Purity of the product checked by TLC showed two distinct spots that were separated by column chromatography. The fractions obtained with 75:25 petroleum ether and ethylacetate mixture as the eluent yielded crystals of 4 suitable for single-crystal X-ray diffraction studies after recrystallization from a MeOH/CHCl₃ solvent mixture. Another product obtained by using 85:15 petroleum ether/ethylacetate mixture as the eluent resulted in the complex 4a and was found to be identical with that of previous reaction product 3a.

Yield: 48%. Colour: Yellow; mp: 190 °C. Anal. Found (%) for C₄₈H₃₉N₂O₂P₂Cu (Mol wt = 801.32): C, 71.52; H, 4.79; N, 3.12. Calculated (%): C, 71.94; H, 4.91; N, 3.50. Selected IR bands (ν max in cm⁻¹): 1582 & 1507 (C=N–N=C); 1362 (enolic C–O); 1084 (N–N). UV-visible (DMSO-buffer): λ max (nm): 215 & 293 (ILCT); 394 (LMCT). ¹H
NMR (500 MHz, CDCl\textsubscript{3}): \( \delta \) (ppm): 8.51 (s, 1H, CH=N); 7.15-7.45 (m, 38H, Ar–H).

**DFT studies**

Quantum chemical investigations on the complex 4 have been carried out by applying the density functional theory (DFT) employing Becke’s three parameter hybrid exchange functional (B3)\textsuperscript{36} combined with correlation functional of Vosko, Wilk and Nusair (VWN)\textsuperscript{37} and Lee, Yang and Parr (LYP),\textsuperscript{38} together called as B3LYP. The effective core potential (ECP) basis set SRSC\textsuperscript{39} for Cu atom and 6-311G(d,p) basis set for all other atoms have been used. The vibrational frequency calculation has been performed at the above level of theory and the results confirm that the optimized geometry is at stationary point of the potential energy surface without any imaginary frequency. Theoretical calculations have been performed by using Q-Chem 3.0 program package.\textsuperscript{40}

**DNA binding studies**

**Preparation of Tris-hydrochloride buffer**

Tris-hydrochloride buffer (5 mM) and sodium chloride (50 mM) were accurately weighed and made upto 250 mL solution in standard measuring flask using double distilled water. The pH of this solution was adjusted to 7.2 using 1 mM sodium hydroxide solution with the help of pH meter (Eutech instruments) before making upto the mark. This buffer solution was used for DNA studies in the relevant chapters.

**Concentration of DNA**

The concentration of calf thymus DNA was determined using spectrophotometry. Solution of CT DNA in Tris-HCl gave ratios of UV absorbance of about 1.8–1.9:1 at 260 and 280 nm, indicating that the CT DNA was sufficiently free of protein.\textsuperscript{41} The CT DNA concentration per nucleotide was determined spectrophotometrically by employing an extinction coefficient of 6600 M\textsuperscript{-1} cm\textsuperscript{-1} at 260 nm.\textsuperscript{42} Similar method was adopted to find out the concentration of DNA in the other chapters wherever the DNA binding studies were performed. The stock solution of the complexes were prepared by dissolving the respective complexes using DMSO and the suitable concentration required for binding experiments were prepared using Tris buffer.
Electronic absorption experiments

Electronic absorption titrations were performed with a fixed concentration of test compounds 3 or 4 (25 μM) but by varying nucleotide concentration from 0 to 25 μM. The absorption band of the compounds 3 and 4 that have undergone significant shift due to the addition of CT DNA was chosen to monitor as an indication of binding between them. From the absorption titration data, the intrinsic binding constant ($K_b$) of the test compounds with CT DNA was determined using the equation,\(^{43}\)

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

where, [DNA] is the concentration of DNA, $\varepsilon_a$, $\varepsilon_f$ and $\varepsilon_b$ are, the apparent extinction coefficient, the extinction coefficient for free test compounds and the extinction coefficient for the test compound in the fully bound form, respectively. A plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs [DNA] gives the intrinsic binding constant $K_b$ as the ratio of slope to the intercept.

Emission experiments

Fixed amounts (25 μM) of the complexes were titrated with increasing amounts of CT DNA, over a range of DNA concentrations from 0 to 25 μM. Excitation wavelength of the complexes 3 and 4 was 288 and 293 nm respectively with the scan speed 200 nm/min, and slit width of 5 nm. After adding DNA to the metal complexes, the emission readings were noted. All experiments were carried out at room temperature in Tris-HCl buffer solution.

Competitive binding experiments

The apparent binding constant ($K_{app}$) of the test compounds 3 and 4 were determined by fluorescence spectral technique using ethidiumbromide (EB) bound CT DNA solution in Tris-HCl buffer (pH, 7.2). Changes observed in the fluorescence intensities at 605 nm (excitation at 545 nm) of EB bound to DNA were measured as a function of concentration of test compounds 3 and 4 (1-100 μM). EB was non-emissive in Tris-HCl buffer solution (pH, 7.2) due to fluorescence quenching of the free EB by the solvent molecules. In the presence of DNA, EB showed enhanced emission intensity due to its intercalative binding to DNA. A competitive binding of the compounds to CT DNA
resulted in the displacement of the bound EB, thereby decreasing its emission intensity. The quenching constant \( K_q \) was calculated using the classical Stern-Volmer equation:\(^{44}\)

\[
\frac{I_0}{I} = K_q [Q] + 1
\]

where, \( I_0 \) is the emission intensity in the absence of quencher, \( I \) is the emission intensity in the presence of quencher, \( K_q \) is the quenching constant, \( [Q] \) is the quencher concentration (\( \mu \text{M} \)) and \( K_q \) is the slope, obtained from the plot of \( [Q] \) vs \( I_0/I \). The apparent binding constant \( (K_{app}) \) has been calculated from the equation,

\[
K_{EB} [EB] = K_{app} \text{[compounds]}
\]

where, \( [EB] = 10 \mu \text{M} \) and \( K_{EB} = 1 \times 10^7 \text{M}^{-1} \).

**Antioxidant studies**

The DPPH, OH and NO radical scavenging activities of the test compounds were determined by using the methods described by Blois, Nash and Green et al respectively.

**DPPH radical scavenging activity**

The DPPH (2-2’-diphenyl-1-picrylhydrazyl) radical scavenging activity of the compounds was measured according to the method of Blois.\(^{45}\) The DPPH radical is a stable free radical having \( \lambda_{max} \) at 517 nm. A variable concentration of the test compounds 1-4 was added to a solution of DPPH in methanol (125 \( \mu \text{M}, 2 \text{mL} \)) and the final volume was made up to 4 \( \text{mL} \) with double distilled water. The solution was incubated at 37 °C for 30 min in dark. The decrease in absorbance of DPPH was measured at 517 nm. The same experiment carried out without the test compounds serve as a control.

**Hydroxyl radical scavenging activity**

The hydroxyl (OH) radical scavenging activity of compounds 1-4 have been investigated using the Nash method.\(^{46}\) *In vitro* hydroxyl radicals were generated by \( \text{Fe}^{3+} / \text{ascorbic acid} \) system. The detection of hydroxyl radicals was carried out by measuring the amount of formaldehyde formed from the oxidation reaction with DMSO. The formaldehyde produced was detected spectrophotometrically at 412 nm. A mixture of 1.0 \( \text{mL} \) of iron-EDTA solution (ferrous ammonium sulphate (0.331 mM) and EDTA (0.698 mM), 0.5 \( \text{mL} \) of EDTA solution (0.048 mM) and 1.0 \( \text{mL} \) of DMSO (10.83 mM) DMSO (v/v) in 0.1 M phosphate buffer, pH 7.4) were sequentially added to the test
tubes containing the test compounds with different concentrations in the range of 10-50 µM. The reaction was initiated by adding 0.5 mL of ascorbic acid (1.25 mM) and incubated at 80-90 °C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1.0 mL of ice cold Trichloroacetic acid (TCA) (107 mM). Subsequently, 3.0 mL of Nash reagent was added to each tube and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the colour formed was measured spectrophotometrically at 412 nm against reagent blank.

**Nitric oxide radical scavenging activity**

Nitric oxide (NO) radical scavenging activity was determined based on the reported method, where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete in this process leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with the test compounds of different concentrations in the range of 10-50 µM and incubated at room temperature for 150 min. The same reaction mixture without the sample but the equivalent amount of the solvent served as the control. After the incubation period, 0.5 mL of Griess reagent containing sulfanilamide (5.8 mM), H₃PO₄ (20 mM) and N-(1-naphthyl) ethylenediamine dihydrochloride (0.39 mM) was added. The absorbance of the chromophore formed was measured at 546 nm.

For each of the above assays, the tests were run in triplicate and various concentrations of the complexes were used to fix a concentration at which each complex showed approximately 50% activity. The percentage activities were calculated using the formula,

\[
\% \text{ activity} = \left(\frac{A_0 - A_C}{A_0}\right) \times 100
\]

where, \(A_0\) and \(A_C\) represent the absorbance in the absence and presence of the tested complex, respectively. The 50% activity (IC₅₀) can be calculated using the percentage of activity results.
Results and discussion

The reactions of $[\text{CuCl}_2(\text{PPh}_3)_2]$ in equimolar amounts with hydrazone ligand either benzoic acid benzylidene-hydrazide (HL$^1$) (1) or benzoic acid furan-2-ylmethylene-hydrazide (HL$^2$) (2) yielded complexes of the type $[\text{Cu}(\text{L}^1)(\text{PPh}_3)_2]$ (3) and $[\text{Cu}(\text{L}^2)(\text{PPh}_3)_2]$ (4) (scheme 2.1). The analytical data of the complexes are in good agreement with the proposed molecular formulae with 1:1 metal to ligand stoichiometry (presented under the experimental part). Both the complexes are quite stable in air and light and soluble in most of the organic solvents such as MeOH, EtOH, CH$_2$Cl$_2$, CHCl$_3$, DMF and DMSO. All the synthesised compounds 1-4 were well characterised using several physico-chemical techniques.

Infrared spectra

The infrared spectra of the free hydrazone ligands 1 and 2 showed some characteristic absorption bands at 3185/3245, 1644/1642, 1546/1539 and 1070/1072 cm$^{-1}$ due to $\nu$(N–H), $\nu$(C=O), $\nu$(C=N) and $\nu$(N–N) stretching vibrations, respectively. But, the corresponding Cu(I) complexes 3 and 4, did not show the band at 3185/3245 cm$^{-1}$ indicating the enolisation of NH group prior to coordination. Also, there appeared two new bands in the range of 1507-1583 cm$^{-1}$ assigned to $\nu$(C–N$^-$N=C) group of them, respectively. The disappearance of $\nu$(C=O) at 1644/1642 cm$^{-1}$ and the appearance of a new band due to $\nu$(C–O) at 1355/1362 cm$^{-1}$ respectively in the Cu(I) complexes suggested that the bonding of the hydrazone to the metal ion was through the deprotonated C–OH group.$^{48}$ Further, $\nu$(N=N) observed at 1070 and 1072 cm$^{-1}$ in the spectra of free ligands 1 and 2 were shifted to higher wavenumber by 12-14 cm$^{-1}$ in the complexes, proving the coordination of one of the nitrogen atom of the N–N group to the metal ion.$^{49}$ The vibrational frequencies calculated from DFT for the complex 4 are in agreement with the experimental results after regular scaling of 0.98.

Electronic spectra

The electronic spectra of the ligands and its complexes were recorded in DMSO-buffer solution. The spectra of the ligands 1 and 2 displayed two bands in the 231-335 nm regions due to intra ligand charge transfer transitions. The electronic spectra of the
complexes 3 and 4 exhibited three bands in the range of 200-400 nm. Bands that appeared in the region of 215, 288 and 293 nm have been assigned to the $\pi \rightarrow \pi^*$ intraligand charge transfer transitions and a weak band at 371 and 394 nm corresponding to the ligand to metal charge transfer transition (LMCT).

**Photoluminescence spectra**

The luminescence behavior of the ligands 1 and 2 and their complexes 3 and 4 were also investigated. When an excitation wavelength corresponding to the lowest energy absorption was used, none of the complexes exhibited emission. However, the use of a higher energy excitation wavelength resulted in an intense emission in each case. The emission maxima for the ligands 1 and 2 appeared at 340 nm and upon coordination with $[\text{CuCl}_2(\text{PPh}_3)_2]$, there observed a red shift for the complex 3 and a blue shift for the complex 4 at the emission maxima of 348 and 337 nm, respectively indicates that this emission originates from the ligand to metal charge transfer (LMCT) state.

**$^1$H NMR spectra**

The $^1$H NMR spectra of both the hydrazone ligands 1 and 2 exhibited a broad signal at 9.2 ppm attributed to the N–H protons. A sharp singlet appeared at 8.1 and 8.2 ppm are assigned to azomethine protons (CH=N) and a multiplet in the range of 7-8 ppm are assigned to the aromatic protons of the phenyl and furyl moieties of the ligands. Upon complexation, the disappearance of the signals at 9.2 ppm due to the N–H
protons indicates that these protons underwent deprotonation prior to the coordination with the metal ion. In addition, the azomethine protons were deshielded and appeared at 8.3 and 8.5 ppm for 3 and 4, suggesting the participation of azomethine nitrogen (CH=N) in coordination with the metal ion (Figs. 2.1 and 2.2). In addition, multiplets in the range of 7-8 ppm were also appeared that are due to the aromatic protons of the phenyl and furyl moieties of the complexes.

**Cyclic voltammetry**

The electrochemical properties of the ligands and copper complexes were studied at a scan rate of 50 mVs\(^{-1}\), in which the ligands are neither reduced nor oxidized reversibly in the applied potential range indicates that the redox processes are assigned exclusively to the metal centered only. The cyclic voltammogram of the complex 3 exhibited an irreversible oxidation at +0.6289 V which is attributed to Cu(I)→Cu(II) and an irreversible reduction peak at -0.8729 V which is assigned to the reduction of Cu(I)→Cu(0). Peaks corresponding to the potentials +0.7676 V and +0.3866 V were obtained for complex 4. The anodic response detected at +0.7676 V is believed to be due to Cu(I)→Cu(II) oxidation and the cathodic response at +0.3866 V is attributed to Cu(II)→Cu(I) reduction and the its large peak to peak separation value (ΔE_p = 381 mV) reveals that the process can be best regarded as a quasi-reversible oxidation. Also, there appeared an irreversible reduction peak at -0.8823 V, attributed to the Cu(I)→Cu(0) reduction process. This behaviour has been reported for related complexes, thus confirms the occurrence of a slow chemical reaction pursuant to the electrode process.\(^{50}\)
X-ray crystallography

Crystallographic study of \([\text{Cu}(L^2)(\text{PPh}_3)_2]\) (4)

From the elemental analyses, IR, electronic and \(^1\)H NMR spectroscopic studies it is understood that the complexes 3 and 4 are structurally similar to each other. One of the monovalent hydrazone complex have been characterised by single crystal X-ray diffraction study. The Cu(I) complex 4 crystallized in the monoclinic lattice with \(P2_1/n\) space group (X-ray diffraction data shown in Table 2.1) with four molecules in an unit cell. The crystal structure presented in Fig. 2.3 showed the composition of the complex 4 as \([\text{Cu}(L^2)(\text{PPh}_3)_2]\), in which the central metal ion was found to be in the 1+ oxidation state instead of the 2+ oxidation state as in the case of the starting precursor. The hydrazone ligand 2 was found to coordinated to the copper ion in the complex 4 in a bidentate fashion through the azomethine nitrogen and enolic oxygen atoms, forming a stable five-member chelate ring with \([\text{P}(1)–\text{Cu}(1)–\text{P}(2)]\), \([\text{P}(1)–\text{Cu}(1)–\text{O}(2)]\), \([\text{O}(2)–\text{Cu}(1)–\text{N}(1)]\) and \([\text{N}(1)–\text{Cu}(1)–\text{P}(2)]\) bite angles of 121.99(5)°, 104.3(1)°, 77.5(1)° and
112.9(1)° respectively with the bond distances of Cu1–P1, Cu1–P2, Cu1–O2 and Cu1–N1 as 2.235(1), 2.253(1), 2.112(3) and 2.044(4) Å, respectively (Table 2.2). Also, the minor product 3a obtained was crystallised to get suitable crystals for characterization using XRD. The single crystal XRD data proved that the molecular formula of 3a is [CuCl(PPh₃)₃] with lack of coordinated hydrazone ligand. Similarly, product 4a was also obtained with that of the complex 4 that are found to be identical with 3a. Hence, it is understood that during the course of the reaction between the starting precursor and the hydrazone ligand, the copper ion gets reduced to a monovalent species in which the hydrazone acted as a good reducing agent. Fig. 2.4 represents the crystal structure of the complex 3a. The crystal structure, unit cell parameters, bond lengths of 3a was found to be in good agreement with the earlier report on the same complex from a different reaction.³⁵

Fig. 2.4 Molecular structure of the complex 3a showing the atom-numbering scheme with thermal ellipsoids at 25% probability level.
Table 2.1 Crystal structure data of the complex 4.

<table>
<thead>
<tr>
<th>Description</th>
<th>Complex 4</th>
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<tr>
<td>Empirical formula</td>
<td>C₃₀H₃₀N₆O₆P₂Cu</td>
</tr>
<tr>
<td>Formula weight</td>
<td>801.32</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P2₁/n</td>
</tr>
<tr>
<td>Temperature</td>
<td>293 K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td></td>
</tr>
<tr>
<td>a (Å)</td>
<td>16.2700(8)</td>
</tr>
<tr>
<td>b (Å)</td>
<td>15.2609(7)</td>
</tr>
<tr>
<td>c (Å)</td>
<td>17.1000(9)</td>
</tr>
<tr>
<td>α (°)</td>
<td>90.000</td>
</tr>
<tr>
<td>β (°)</td>
<td>107.274(3)</td>
</tr>
<tr>
<td>γ (°)</td>
<td>90.000</td>
</tr>
<tr>
<td>Colour</td>
<td>Yellow</td>
</tr>
<tr>
<td>D&lt;sub&gt;c&lt;/sub&gt; (Mg/m&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>1.313</td>
</tr>
<tr>
<td>F(000)</td>
<td>1664</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
<tr>
<td>Crystal size (mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>0.11 x 0.16 x 0.20</td>
</tr>
<tr>
<td>hkl limits</td>
<td></td>
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<tr>
<td>0 &lt;= h &lt;= 16</td>
<td></td>
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<tr>
<td>-15 &lt;= k &lt;= 15</td>
<td></td>
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<tr>
<td>-17 &lt;= l &lt;= 17</td>
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<tr>
<td>0 range for data collection</td>
<td>1.50 to 21.30°</td>
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<tr>
<td>Reflections collected</td>
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<tr>
<td>No. indep. Reflns</td>
<td>4507</td>
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<tr>
<td>R&lt;sub&gt;int&lt;/sub&gt;</td>
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<tr>
<td>R indices (all data)</td>
<td>R1 = 0.0433, wR2 = 0.1228</td>
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<tr>
<td>Goodness-of-fit (F&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1.07</td>
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Table 2.2 Comparison of some selected bond lengths (Å) and bond angles (°) using XRD and theoretical data of the complex 4.

<table>
<thead>
<tr>
<th>Selected bond lengths</th>
<th>XRD</th>
<th>Theoretical</th>
<th>Selected bond angles</th>
<th>XRD</th>
<th>Theoretical</th>
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<tr>
<td>Cu₁–P₁</td>
<td>2.235(1)</td>
<td>2.341</td>
<td>P₁–Cu₁–P₂</td>
<td>121.99(5)</td>
<td>121.349</td>
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<tr>
<td>Cu₁–P₂</td>
<td>2.253(1)</td>
<td>2.352</td>
<td>O₂–Cu₁–P₂</td>
<td>110.3(1)</td>
<td>102.236</td>
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<tr>
<td>Cu₁–O₂</td>
<td>2.112(3)</td>
<td>2.139</td>
<td>N₁–Cu₁–P₂</td>
<td>112.9(1)</td>
<td>114.925</td>
</tr>
<tr>
<td>Cu₁–N₁</td>
<td>2.044(4)</td>
<td>2.121</td>
<td>P₁–Cu₁–O₂</td>
<td>104.3(1)</td>
<td>109.043</td>
</tr>
<tr>
<td>O₂–C₇</td>
<td>1.262(7)</td>
<td>1.270</td>
<td>N₁–Cu₁–O₂</td>
<td>77.5(1)</td>
<td>76.043</td>
</tr>
<tr>
<td>C₇–N₂</td>
<td>1.317(6)</td>
<td>1.335</td>
<td>N₁–Cu₁–P₁</td>
<td>119.1(1)</td>
<td>119.888</td>
</tr>
<tr>
<td>N₂–N₁</td>
<td>1.393(6)</td>
<td>1.368</td>
<td></td>
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</tbody>
</table>

DFT Calculations

The DFT calculations using the hybrid exchange correlation functional B3LYP have been performed on the related mononuclear species [Cu(L<sup>2</sup>)(PPh₃)₂] (4) with the ground-state geometry adopted from the truncated X-ray data. After careful comparison, we found that most of the optimized bond lengths and angles are slightly larger than the
experimental values, which is not surprising due to the known fact that theoretical calculation belongs to the isolated molecule in the gaseous phase and the experimental result belongs to the molecule in the solid state with intermolecular interactions and crystal packing effect. The optimized structure of the stable conformer by DFT method for the complex 4 is shown in Fig. 2.5. Some selected single crystal X-ray diffraction data together with the optimized geometrical parameters at the B3LYP method are listed in Table 2.2.

To understand the nature of electronic transitions, the molecular orbital analysis of the complex 4 has been performed which is comparable to that of experimentally observed absorption spectral data. Fig. 2.6 represents the frontier molecular orbital’s of the complex 4, i.e., the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO), in which HOMO is mainly delocalized on imine group and the LUMO is mainly delocalized over the imine as well as in aromatic group with an energy gap of 3.54 eV between the frontier orbitals.

![Fig. 2.5 Optimized ground-state structure of the complex 4 at the B3LYP/6-311G(d,p) level.](image)
Metal complexes are known to bind with DNA via both covalent and non-covalent interactions. In covalent binding the labile ligand of the complexes is replaced by a nitrogen base of DNA such as guanine N7. On the other hand, non-covalent DNA interactions include intercalative, electrostatic and groove (surface) binding of cationic metal complexes along outside of DNA helix, along major or minor groove. Intercalation involves the partial insertion of aromatic heterocyclic rings of the ligands between the DNA base pair. In this study, we selected complexes 3 and 4 to investigate the binding properties with DNA which can be monitored through UV-visible and fluorescence studies.

Generally, the binding of an intercalative molecule to DNA is always accompanied by hypochromism and / or significant bathochromism in the absorption spectra due to strong stacking interactions between the aromatic chromophore of the compounds and DNA base pairs. To obtain some conclusive proof for the binding of the test complexes with DNA, spectroscopic titrations of complex solutions with DNA have been performed.
The absorption spectra of the investigated metal complexes in the absence and presence of DNA are given in Fig. 2.7. In the absence of DNA, the absorption spectra of the complex 3 displayed three bands around 215, 288 and 371 nm. Similarly, the complex 4 exhibited absorptions at 215, 293 and 394 nm. But, upon the addition of DNA to the solution of the metal hydrazone complexes 3 and 4, the bands observed at 215 nm for both the complexes exhibited a respective hypochromism of about 5.59% and 22.54% accompanied by a red shift of 4 and 6 nm corresponding to them. However, in the case of complex 3, the band appeared at 288 nm showed an increase in the absorption intensity only after the addition of the first fraction of DNA solution, whereas further additions resulted in 9.99% hypochromism and the band observed at 371 nm showed a hyperchromism of about 18%. Absorptions appeared at 293 and 394 nm for the complex 4 exhibited a hypochromism of 6.9% with respect to the former band and a hyperchromism of 12.91% corresponding to the later absorption. The intrinsic binding constant (Kₘ), was calculated according to the classical equation and the Kₘ values obtained for the complexes 3 and 4 were found to be 2.05×10⁴ M⁻¹ and 1.80×10⁵ M⁻¹, respectively revealing that the complex 4 exhibited more intense hypochromism with higher Kₘ value than the complex 3, indicating a stronger interaction exists between CT DNA and complex 4 via groove binding.⁴³

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**Fig. 2.7** Electronic absorption spectra of complexes 3 and 4 (25 μM) in the absence and presence of increasing amounts of CT DNA (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5 and 20.0, 22.5 and 25 μM; subsequent spectra). Arrows show the absorbance changes upon increasing DNA concentration. (Inset: A plot of [DNA]/(ε_a - ε_f) vs [DNA]).
Emission measurements

Complexes 3 and 4 in DMSO-Tris-HCl buffer upon excitation with 288 and 293 nm wavelength at room temperature showed luminescence emission at 348 and 337 nm, respectively and the results of the emission titration for the complexes added with DNA is represented in Fig. 2.8. Upon addition of calf thymus DNA, the emission intensity increases to 6.64% and 11.35%, respectively when [DNA]/[complex] = 1.

![Emission spectra of complexes 3 and 4](image)

Fig. 2.8 The emission enhancement spectra of complexes 3 and 4 (25 µM) in the absence and presence of increasing amounts of CT DNA (2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0, 22.5 and 25 µM). Arrows show the emission intensity changes upon increasing DNA concentration.

Competitive binding measurements

Further, to know the interaction of the complexes 3 and 4 with DNA, a competitive binding experiment using ethidium bromide (EB) as a probe was carried out and the fluorescence quenching of EB bound to CT DNA by the above complexes are shown in Fig. 2.9. The addition of complexes 3 and 4 to EB-bound CT DNA solution caused obvious reduction in emission intensities with 3 nm blue shift (complex 3) and 3 nm red shift (complex 4), indicating that complexes competitively bound to CT DNA and caused release of bound EB from the hydrophobic environment into the aqueous solution or accepting an excited state electron from EB. The emission intensity of the DNA-EB system (λ_{em} = 605 nm) decreased apparently as the concentration of the complexes increased. For complex 4, an isobathic point appeared at 549 nm, indicated the formation of a complex-DNA system. According to the equation, K_{EB} [EB] = K_{app}[complex],
(where the complex concentration has the value at a 50% reduction of the fluorescence intensity of EB, K_{EB} = 1.0 \times 10^7 \text{ M}^{-1} \text{ and } [EB] = 10 \mu \text{M}) the K_{app} value was calculated to be 8.42 \times 10^4 \text{ M}^{-1} and 1.16 \times 10^5 \text{ M}^{-1} for complexes 3 and 4, respectively, from the plot of I_{0}/I vs [complex] (as insets in Fig. 2.9) suggesting that the interaction between the above mentioned complexes and DNA is through moderate groove binding. The quenching constants K_q are often used to evaluate the quenching efficiency for every compound and vary with experimental conditions. K_q values are given by the ratio of the slope to the intercept and concentration of the corresponding complexes and are found to be 2.28 \times 10^3 \text{ M}^{-1} and 6.55 \times 10^3 \text{ M}^{-1}, respectively. Experimental results indicated that the complex 4 bound to DNA more effectively than the complex 3 which may be due to the presence of heterocyclic moiety, i.e., furan ring.

**Fig. 2.9** Emission spectra of DNA-EB system (10 µM), in the presence of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µM complexes 3 and 4. Arrow indicates the emission intensity changes upon increasing complex concentrations. (Inset: Stern-Volmer plot of the fluorescence titration data corresponding to the complexes 3 and 4).

**Antioxidant activity**

Based on the known fact that hydrazones and their corresponding transition metal complexes displayed significant antioxidant activity, we undertook a systematic investigation on the antioxidant potential of the ligands 1 and 2 and their complexes 3 and 4 on DPPH•, OH• and NO• at various concentrations. The results of these experiments are shown in Fig. 2.10. IC_{50} values of the ligands 1 and 2 on DPPH, OH and NO radicals are 62.42, 60.02, 59.59, 61.14, 58.84 and 57.18 µM, respectively, whereas,
the complexes 3 and 4 showed their IC\textsubscript{50} values at 52.47, 46.18, 42.14, 50.77, 41.21 and 37.29 μM, respectively. From the above results, it can be concluded that the scavenging effects of the free ligands are less when compared to that of their corresponding complexes which is due to the chelation of the organic molecules with the metal ions. However, among the two investigated complexes, the one containing furan moiety (complex 4) in their molecular architecture showed higher antioxidant activity on DPPH\textsuperscript{•}, OH\textsuperscript{•} and NO\textsuperscript{•} than the other (complex 3) containing phenyl ring. The overall scavenging activity of the tested compounds was found to decrease in the order of 4 > 3 > 2 > 1. Further, the results obtained against the three different radicals confirmed that the complexes are more effective to arrest the formation of the nitric oxide than the hydroxyl and DPPH radicals and the lower IC\textsubscript{50} values observed in antioxidant assays did demonstrate that these complexes exhibited differential and selective effects to scavenge radicals and hence the potential as drugs to eliminate the radicals.

![Fig. 2.10 Scavenging effects of ligands 1, 2 and its complexes 3, 4 on DPPH\textsuperscript{•}, HO\textsuperscript{•} and NO\textsuperscript{•}.](image-url)
Conclusion

In this present study, a set each of univalent copper hydrazone complexes have been synthesized from the reactions of \([\text{Cu(Cl}_2\text{)(PPh}_3\text{)}_2]\) and hydrazones 1 and 2 in 1:1 stoichiometric ratio and characterised using different physico-chemical techniques including single crystal XRD. The density functional theory calculations of the complex 4 also supported the structure and stability of the reduced complex. The results of biological experiments showed that the metal complexes 3 and 4 showed significant binding towards calf thymus DNA (CT DNA) via groove binding mode with binding constants in the magnitude of \(10^4-10^5\ M^{-1}\). In addition, the antioxidant activities of all the synthesised compounds investigated on DPPH\(^-\), NO\(^-\) and OH\(^-\) showed that the compounds are potential antioxidants.
Reference


