Short communication

DNA interaction and cytotoxic activity of copper complex based on tridentate hydrazone derived ligand and nitrogen donor heterocycle

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A B S T R A C T
Tetra coordinated copper(II) complex of formula [Cu(L1)(imid)] is synthesized using imidazole and a tridentate O,N,O hydrazone ligand L1 prepared by condensation of 1,1,1-trifluoropentanedione and 4-chlorobenzhydrazide. The ligand and complex are characterized by UV–Visible, FTIR, NMR, mass and single crystal XRD techniques. DNA binding mode is assessed by UV absorption, fluorescence spectral and circular dichroism studies. Based on the results, it is observed that complex has preferred intercalative mode of binding with DNA. The binding constant, \( K_b \), was found to be \( 4.02 \pm 0.09 \times 10^4 \) M\(^{-1} \). Thermodynamic parameters such as \( \Delta H \), \( \Delta S \) and \( \Delta G \) obtained from acridine orange fluorescence displacement assay revealed that the hydrophobic and hydrogen bonding interactions are playing a major role in the binding pattern. In addition, cytotoxicity of the complex towards MCF-7 breast cancer cell line has also been assessed.

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Metallodrugs have set a mark in the field of bioinorganic chemistry since the serendipitous discovery of cisplatin by Rosenberg and others [1,2]. Anticancer activities of cisplatin and second generation platinum drugs have been assessed based on their structure activity relations implying the significance of ligand structure towards therapeutic efficacy [3,4]. Hence, the design of new metal complexes is focused on the design of ligand structure and its functionality towards metal center. One way of tuning the selectivity of the drug molecule is to manipulate the ligand environment to suit to specific target. Thus, the nature of the ligand is expected to play an important role in the binding of the metal complex to the biomolecules such as DNA or protein. Thus, complexes with varied coordination number and geometry based on different ligand systems have shown a wide variety of biological activities such as DNA and protein binding [5,6], anticancer [7], antibacterial [8] and antidiabetic [9] activities. DNA is an important target for cytotoxic drugs. The design of DNA targeting metal based compounds have gained much interest because of their impact on structure and conformation of biomolecules thereby affecting cellular processes [10]. Severe side effects and toxicity issues [11–13] related to platinum based drugs have prompted research in bioessential metals such as copper(II), zinc(II), vanadium(IV/V), etc.

Copper, being a necessary trace element, has remarkably emerged as a chemotherapeutic agent under the category of elemental medicines [14]. It constitutes superoxide dismutases (SODs) as well as cytochrome C oxidase which are essential for aerobic respiration [15]. The efficiency with which copper complexes bind to DNA is greatly influenced by the nature of coordinating ligand. The various binding modes such as coordinate [16], non-covalent [17] and intercalative [18], partial intercalative [19] with DNA are susceptible to variations based on the nature of ancillary ligand or coordinated ions. In view of this, ligands based on aryl hydrazones are studied extensively for their chemical as well as biological applications [19]. In addition, due to keto-enol tautomerism, they provide neutral [20], mono [21], di [22–24], or tetraanionic [25,26] species leading to unusual coordination numbers with metals. In the current study, copper(II) complex based on tridentate O,N,O hydrazone ligand and an ancillary monodentate nitrogen heterocycle, imidazole, is considered and explored for its DNA binding behavior, hitherto unreported. An attempt has also been made to assess its cytotoxic activity towards breast cancer cell line MCF-7.

In the present work, [Cu(L1)(imid)], complex (Fig. 1) was prepared using equimolar ratios of L1, imidazole and copper(II) acetate monohydrate (Fig. S1) [27,28] and characterized by UV–visible, FTIR and single crystal XRD techniques. Electronic spectra of the ligand and complex were recorded in methanol. UV absorption spectra of the ligand L1 shows intense peaks in the range of 200–400 nm and the sharp peaks at 210 nm and 240 nm assigned to intraligand π–π* transitions (Fig. S2). In the case of complex, peaks are shifted to higher...
of the complex has shown absorption spectral titrations, at 218 nm and 249 nm, respectively. A distinct metal ligand charge transfer (MLCT) is observed at 352 nm in the case of metal complex. The visible spectrum shows a distinct band at 249 nm, respectively. A distinct metal ligand charge transfer (MLCT) is observed at 660 cm$^{-1}$ for the complex.

FTIR spectrum of the ligand L$^1$ (Fig. S3) has shown specific stretching frequency at 1662 cm$^{-1}$ for $\textit{C}=\textit{O}$ group of amide functionality. Imine ($\textit{C}=\textit{N}$') and secondary $\textit{N}H$ group stretching frequencies were observed at 1643 cm$^{-1}$ and 3360 cm$^{-1}$, respectively. The disappearance of carbonyl group and secondary $\textit{N}H$ frequency in the complex is attributed to the enolisation of keto functionality (Fig. S4). The shifting of imine functional group to 1618 cm$^{-1}$ proves its chelation with copper. The characteristic M–N (metal nitrogen) frequency was observed at 660 cm$^{-1}$ in the complex.

In$^1$H NMR spectrum (Fig. S5) of the ligand L$^1$, protons from the free methyl group of diketone appear as a singlet at $\delta$ value 2.05 ppm with integration value 1.54 depicting the presence of three protons. Aromatic protons present in the ring near to the chlorine substitution were observed at $\delta$ value 7.83 ppm and 7.86 ppm with same integration value as they have similar magnetic environments. The remaining two aromatic protons were found to be present at $\delta$ value 7.396 ppm and 7.41 ppm. Geminal coupling was observed for $-\text{CH}_2$ protons at $\delta$ value 3.11–3.32 ppm. On the other hand, $^{13}$C NMR of the ligand (L$^1$) (Fig. S6) has shown the presence of methyl carbon atom at 15.86 ppm and $-\text{CH}_2$ carbon atom at 46.89 ppm. Aromatic carbon atoms having similar magnetic environment were found to be present at $\delta$ value 122.07–131.79 ppm, whereas, carbon atom attached to chlorine was observed to be shifted downfield at $\delta$ value 138.59 ppm. The presence of carbonyl carbon was confirmed at $\delta$ value 170.01 ppm and imine carbon at 155.23 ppm. On the other hand, carbon atom from trifluoromethyl group was confirmed at $\delta$ value 92.49–92.83 ppm.

The molecular weight of the ligand (L$^1$) was confirmed by the GC–MS spectrometry. Molecular ion peak was present at 306.14 m/z value as shown in Fig. S7. The chromatogram has shown a single peak at RT 16.38 min for the respective ligand with area under curve of about 99.19% (Fig. S7). The obtained copper complex crystal [29] has the dimension of 0.300 × 0.200 × 0.150 mm$^3$ and the crystal data (supplementary data) for the same has been presented (Fig. S8).

For the DNA interaction studies, the stability of the complex in Tris HCl buffer was investigated for 2 h duration by UV absorption spectroscopy and no considerable change was observed indicating the stability of [Cu(L$^1$)(imid)] in aqueous solution (Fig. S9).

Interaction of the metal complex with calf thymus DNA (CT-DNA) was investigated by observing the UV spectra of the complex in the presence of increasing amounts of CT-DNA [30]. Hypochromism observed for the complex in the presence of DNA (Fig. 2), is characteristic of intercalative mode of DNA binding. The isosbestic point observed in UV titration curves is indicative of equilibrium between the free and bound forms. The planar hydrazone moiety of the complex may have inserted between the DNA base pairs forming a π–π stacking interaction and thus leads to the changes in the absorbance spectra after the addition of DNA. The binding affinity of the complex $K_b$ was calculated using the formula, $[\text{DNA}] / (\varepsilon_a - \varepsilon_f) = [\text{DNA}] / (\varepsilon_b - \varepsilon_f) + 1 / K_b (\varepsilon_b - \varepsilon_f)$, where, $\varepsilon_a$, $\varepsilon_b$ and $\varepsilon_f$ correspond to $A_{obsd} / [\text{Cu}^{2+}])$, the molar extinction coefficients for free copper complex and that in fully bound form, respectively. [DNA]/($\varepsilon_b - \varepsilon_f$) vs [DNA] values were plotted and $K_b$ was obtained from the ratio of the slope to the intercept. Intrinsic binding constant for complex was found to be $(4.02 \pm 0.09) \times 10^4$ M$^{-1}$ comparable with the reported values [31,32].

The intercalative behavior of the complex was supported by the ethidium bromide displacement assay (Fig. S10). The ability of the complex to displace EtBr was calculated quantitatively using Stern–Volmer constant. $K_s$ was found to be $2.08 \times 10^3$ M$^{-1}$ $(R = 0.9915)$ [33,34].

Circular dichroism study of DNA shows positive band at 273 nm due to the base stacking and negative band at 244 nm due to helicity. In the presence of complex (50 $\mu$M), a slight change in the positive and negative bands was observed. (Fig. S11). But, with increased concentration of the complex (100 $\mu$M), a marked increase in the positive band by 1 nm
was observed, probably, due to perturbations in the base stacking behavior of DNA supporting intercalative binding pattern, while, groove binding mode never alters the base stacking and helical structure of the DNA\[35,36\].

In order to understand the forces involved in the interactions, an acridine orange fluorescence displacement assay (Fig. 3) was carried out at different temperatures [37,38]. A linear Stern–Volmer graph was plotted and $K_{sv}$ values were calculated. The $K_{sv}$ values (Table 1) for the complex at different temperatures (294 K, 304 K and 314 K) suggested the effect of temperature in the quenching mechanism. The steady decrease in the $K_{sv}$ values according to increase in the temperature suggested the static quenching rather than dynamic. The modified Stern–Volmer plot was used to calculate binding constant, $K$, whose values have decreased gradually with the temperature increments. Further, thermodynamic parameters such as enthalpy change ($\Delta H$), entropy change ($\Delta S$) and Free energy change ($\Delta G$) were calculated using Van’t Hoff plot (Fig. 4) and the type of interaction between DNA and complex was predicted. The negative $\Delta H$ value $-2.59$ kcal/mol depicts that the binding interactions are enthalpy driven and mainly involve hydrogen bonding. Positive $\Delta S$ value, 7.96 cal/mol/K suggests the involvement of hydrophobic interactions. Thus, complex binds to DNA through hydrogen bonding as well as hydrophobic interactions [38].

The interaction of the complex with the DNA through hydrogen bond is supported by molecular docking studies. Complex was docked using DNA (PDB ID: 2D55) and was found to get intercalated in the base pairs (Fig. S12) [39,40]. Oxygen atom of the carbonyl group of the complex has shown hydrogen bond of 3.54 A.U. with hydrogen atom of the deoxycytosine (DC13) situated in chain A (Fig. S12). The binding energy of the complex was found to be $-9.5$ kcal/mol.

Ligand and complex were tested against MCF-7 breast cancer cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for the assessment of in-vitro antiproliferative assay. As seen, the viability of cell line decreases in both ligand- and complex-treated ones as a function of concentration indicating a dose-dependent growth (Fig. 5) inhibitory effect. It is clear that the cytotoxic effect (Fig. S13) of the copper(II) complex against MCF-7 is significantly higher than that of the ligand (L1). IC50 value for the copper(II) complex was found to be 5.8 $\mu$M whereas L1 has 31.21 $\mu$M. It is evident that the cytotoxicity of the ligand has improved upon complexation [41]. Further, antiproliferative activity of the complex is significant as compared to reported value of cisplatin (IC50 $= 22.8 \mu$M) on the same cell line [42].

This communication has probed into the synthesis and DNA binding, cytotoxic studies of novel O,N,O tridentate hydrazone ligand L1 and imidazole based tetracoordinated copper(II) complex, [Cu(L1)(imid)]. The synthesized ligand and complex were characterized by UV–visible, FTIR, Mass and Single crystal XRD studies. The complex has shown the intercalative mode of binding towards DNA which is proved by hypochromatic effect in UV titration, quenching effect in the EtBr and AO displacement assay and perturbations in the circular dichroic DNA bands. Thermodynamic parameters suggested the role of hydrophobic and hydrogen bond interactions in the DNA binding process of the complex. Molecular docking studies supported the intercalative nature and the hydrogen bonding behavior with DNA. As per the results obtained

![Fig. 3. Acridine orange displacement assay of complex at different temperatures.](image-url)
from MTT assay on MCF-7 breast cancer cell line, the complex is found to be cytotoxic.

Acknowledgements

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Appendix A. Supplementary material

Crystallographic data for the [Cu(L1)(imid)] has been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 1439423. Copies of this information may be obtained from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44–1223-336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk). Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.inoche.2016.03.012

References


Table 1

Thermodynamic parameters and different binding constants of [Cu(L1)(imid)] obtained from acridine orange fluorescence displacement assay.

<table>
<thead>
<tr>
<th>T</th>
<th>$K_{sv}$ ($×10^3$) (M$^{-1}$)</th>
<th>$K_q$ ($×10^{12}$) (M$^{-1}$ s$^{-1}$)</th>
<th>$K$ ($×10^4$) (M$^{-1}$)</th>
<th>n</th>
<th>$R^2$</th>
<th>$\Delta H$ (kcal·mol$^{-1}$)</th>
<th>$\Delta S$ (cal mol$^{-1}$ K$^{-1}$)</th>
<th>$\Delta G$ (kcal·mol$^{-1}$)</th>
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<tr>
<td>294</td>
<td>2.06</td>
<td>2.06</td>
<td>8.26</td>
<td>0.89</td>
<td>0.9935</td>
<td>$-4.93$</td>
<td>$-5.00$</td>
<td>$-5.08$</td>
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<tr>
<td>304</td>
<td>1.53</td>
<td>1.53</td>
<td>6.22</td>
<td>1.02</td>
<td>0.9941</td>
<td>$-5.00$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>314</td>
<td>0.89</td>
<td>0.89</td>
<td>4.26</td>
<td>1.10</td>
<td>0.9954</td>
<td>$-2.59$</td>
<td>7.96</td>
<td></td>
</tr>
</tbody>
</table>

$R$ is the coefficient of correlation.

Fig. 4. a) Stern–Volmer plot for the calculation of $K_{sv}$ at different temperatures b) Modified Stern–Volmer plot for the calculation of $K$ c) van’t Hoff plot for the calculation of $\Delta H$, $\Delta S$ and $\Delta G$.

Fig. 5. Percentage inhibition of cell growth in the presence of a) complex and b) ligand.
Hot methanolic solutions of 4-chlorobenzhydrazide (2.49 mmol, 500 mg) and methanolic solution of Imidazole (1 mmol, 68 mg) was added and stirred vigorously for 5 min. The reaction mixture was refluxed for 2 h at 65–70 °C. The resultant dark green mixture was filtered and X-ray quality crystals were grown by the diffusion of diethyl ether into a 1:5 mixture of methanol and acetonitrile. [Cu(L1)](mid) = Yield: 72%. Dark green crystal. Anal Calc. For C15H12ClCuF3N4O2: C: 41.30, H: 2.77, N: 12.84. Found: C: 41.23, H: 2.75, N: 12.81. UV–Vis (methanol): λmax (MeOH)/nm (ε, dm−3 mol−1 cm−1): 218(35,650), 249(18,400), 362(16,300), 580(140), FT-IR (KBr, νmax/cm−1): 1618, 660.

S. Das, S. Pal. X-ray diffraction data for the complex was collected using a Bruker AXS kappa APEXII single crystal CCD diffractometer. The structure was solved by direct methods procedure using SHELXS-97 program and the non-hydrogen atoms were subjected to anisotropic refinement by full matrix least squares on F2 using SHELXL-97 program. It shows square planar geometry similar to the reported aroyl hydrazone based copper(II) complex, J. Mol. Struct. (2005) 183–192.

UV absorbance of the commercial calf thymus DNA in a buffer gave an absorption ratio (A260/A280) of about 1.9:1, indicating that the DNA was sufficiently free from protein. The concentration of DNA in nucleobases was determined using molar extinction coefficient of 6600 M−1 cm−1 at λmax 260 nm. All the DNA experiments were carried out in a Tris HCl buffer at pH 7.2 which was prepared in Milli-Q triple deionized water. Absorption spectral titration of complex was recorded in the range of 200–600 nm keeping the complex concentration constant (25 μM) and varying the CT-DNA concentrations from 0–120 μM in 50 mM Tris HCl buffer at pH 7.2. To nullify the absorbance changes because of the DNA, an equal volume of DNA was added to both the reference cell and the sample cells.


Ethidium bromide displacement assay was carried out using DNA–EtrBr complexes including 10 μM EtrBr and 100 μM of CT-DNA concentrations in 50 mM Tris HCl buffer (pH 7.2). The DNA–EtrBr complex was excited at 510 nm and the subsequent emission maxima was observed at 596 nm. The successive increments of complex from 20 to 120 μM was done.


CD spectra were recorded with a Jasco J-7 spectropolarimeter at using 0.1 cm quartz cell. The concentration of CT-DNA (100 μM) was kept constant and the experiment was carried out using the two different concentrations of the complex as 50 μM and 100 μM. The experiment was done in triplicates to get a final spectrum as an average of successive scans. The blank correction was done for each run.

Acridine orange fluorescence displacement assay was carried out using DNA – Acridine orange complexes including 10 μM AO and 100 μM of CT-DNA concentrations in 50 mM Tris HCl buffer (pH 7.2) at different temperatures such as 294 K, 304 and 314 K. The DNA-AO complex was excited at 480 nm and the subsequent emission maxima was observed at 520 nm. Thermodynamic parameters such as ΔH, ΔS and ΔG values were calculated. The successive increments of complex from 25 to 200 μM was done.


Molecular docking studies were carried out using Autodock Vina and MGL Tools 1.5.2. The results were viewed in Pymold Viewer. The grid points of 56 × 54 × 58 with 1.0 Å spacing were calculated around the docking area for all the ligand atom types using default optimization parameters.

