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*Scientific paper*

# Synthesis, Structure Characterization, DNA Binding, and Cleavage Properties of Mononuclear and Tetranuclear Cluster of Copper(II) Complexes

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## Abstract

Two copper(II) complexes, cluster **1,** and mononuclear **2,** have been synthesized by reacting acetylacetone and benzohydrazide (1:1 ratio for **1** and 1:2 ratio for **2**) with $CuCl_2$ in a methanol solution. In **2**, which is a new complex, the ligand acts as a tetradentate which binds the metal ion via two amide-O atoms and two imine-N atoms providing an $N_2O_2$ square-planar around the copper(II) ion. The absorption spectra data evidence strongly suggested that the two copper(II) compounds could interact with CT-DNA (intrinsic binding constant, $K_b = 0.45 \times 10^4$ $M^{-1}$ for **1** and $K_b = 2.39 \times 10^4$ $M^{-1}$ for **2**). The super coiled plasmid pBR322 DNA cleavage ability was studied with **1** and **2** in the presence and absence of $H_2O_2$ as an oxidant. In both the absence and the presence of an oxidizing agent, complex **2** exhibited no nuclease activity. However, even in the absence of an oxidant, complex **1** exhibited significant DNA cleavage activity.

**Keyword:** copper complex; mononuclear; copper cluster; DNA binding; DNA cleavage

## 1. Introduction

Recently, there has been considerable interest in the interaction of transition metal complexes with DNA and nuclease activity studies because of their various applications in nucleic acid chemistry. The use of such complexes in foot-printing studies, as sequence specific DNA binding agents, as diagnostic agents in medicinal applications, and for genomic research, has generated the current interest to further develop this chemistry.[1–4] The first report of a synthetic Cu(II) system capable of inducing DNA cleavage was given by Sigman *et al.* in 1979.[5] Afterwards, large numbers of copper(II) complexes and other metal complexes, particularly of the first row transition metals, have been synthesized, characterized, and evaluated for their nuclease mimicking and anticancer activities.[6–8] The two modes of binding with DNA residues have been identified, i.e., intercalating and covalent binding. The former requires planar type structures while the latter needs coordination complexes with potential coordination sites.[9, 10] There are three different types of DNA cleavage mechanisms, *viz* oxidative cleavage, photochemical cleavage, and hydrolysis.[11,12] Copper(II) complexes are capable of binding and cleaving double-stranded DNA under physiological conditions.[13,14]

For these reasons, we have been systematically studying the DNA cleavage and nuclease activity of various copper(II) complexes. Our group began to synthesize ligands derived from benzohydrazide and their copper complexes and to evaluate their DNA binding and cleavage abilities. In this paper, we describe the synthesis, characterization by elemental analysis, FT–IR, UV–Vis and X-ray crystallography, and DNA binding and cleavage abilities of a copper(II) complex with tetradentate ligand derived from a benzohydrazide and tetranuclear copper(II) cluster $[Cu_4Cl_6O(C_5H_8N_2)_4]$.

## 2. Experimental

### 2. 1. Reagents

All chemicals were used as supplied by Merck and Fluka without further purification. Calf thymus DNA (CT-DNA) and supercoiled pBR322 DNA were obtained from Sigma–Aldrich and stored at 4°C. The Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer was prepared in doubly distilled water.

### 2. 2. Physical Measurements

Infrared spectra were taken with an Equinox 55 Bruker FT-IR spectrometer using KBr pellets in the 400–4000 $cm^{-1}$ range. Absorption spectra were determined in the solvent methanol using a GBC UV-Visible Cintra 101 spectrophotometer with 1 cm quartz, in the range of 200–800 nm at 25 °C. Elemental analyses (C, H, N) were performed using a CHNS-O 2400II PARKIN-ELMER elemental analyzer.

### 2. 3. DNA Binding

The interaction of the complexes with calf thymus CT-DNA was studied in the Tris–HCl buffer (5.0 mM Tris–HCl, pH 7.2) containing 50mM NaCl at room temperature. Then, the solution was kept for over 24 h at 4 °C. The resulting somewhat viscous solution was clear and particle-free. The CT-DNA in the buffer medium gave a ratio of UV absorbance at 260 and 280 nm of 1.8:1, indicating that the DNA was sufficiently free of protein.[15] The DNA concentration was measured from its absorption intensity at 260 nm using the molar absorption coefficient (ε) value of 6600 $M^{-1}$ $cm^{-1}$ as reported.[16] The stock solutions of complexes were freshly prepared by first dissolving complexes in DMSO for **1** and in DMF for **2**, then diluting them with the buffer. The amount of DMSO or DMF was kept at 10% (by volume) for each set of experiments and had no effect on any experimental results. After equilibrium reached (ca. 5 min) the spectra were recorded against an analogous blank solution containing the same concentration of DNA. Absorption spectral titration experiments were performed while maintaining a constant complex concentration and varying the nucleic acid concentration. This was achieved by dissolving an appropriate amount of the metal complex (40 μM for **1** and 70 μM for **2**) and DNA stock solutions (0–60 μM) while maintaining the total volume constant (1 ml). The spectral bands exhibited hyperchromism for **1** and hypochromism for **2** in each investigated complexes and were recorded after the successive addition of CT-DNA. The Tris–HCl buffer was used as a blank to make preliminary adjustments. The intrinsic binding constant ($K_b$) of the complexes to CT-DNA were determined from the spectral titration data using the following equation.[17]

**Table 1.** Crystallographic data of the complexes **1** and **2**

| Compound | 1 | 2 |
|---|---|---|
| Chemical formula | $C_{20}H_{32}Cl_6Cu_4N_8O$ | $C_{19}H_{16}CuN_4O_3$ |
| Formula weight | 867.43 | 411.91 |
| Temperature (K) | 200 | 200 |
| Space group | Monoclinic, $p_{2/n}$, Z=2 | Orthorhombic, pbca, Z = 8 |
| Unit cell dimensions | | |
| a (Å) | 13.3604 (3) | 19.8983 (10) |
| b (Å) | 8.8530 (1) | 8.4055 (3) |
| c (Å) | 16.6709 (4) | 20.8417 (10) |
| α (°) | 90 | 90 |
| β (°) | 95.0682 (9) | 90 |
| γ (°) | 90 | 90 |
| V ($Å^3$) | 1964.12 (7) | 3485.9 (3) |
| F(000) | 868 | 1688 |
| $D_{Calc}$ (g $cm^{-3}$) | 1.467 | 1.570 |
| Crystal size (mm) | 0.44 × 0.10 × 0.07 mm | 0.54 × 0.04 × 0.01 mm |
| μ ($mm^{-1}$) | 2.57 | 1.28 |
| θ range (°) | 2.6–27.5 | 2.8 – 25 |
| Limiting indices | –17 ≤ h ≤ 17 | –19 ≤ h ≤ 23 |
| | –11 ≤ k ≤ 11 | –10 ≤ k ≤ 9 |
| | –21 ≤ l ≤ 20 | –24 ≤ l ≤ 24 |
| R[F2 > 2σ(F2)] | 0.043 | 0.055 |
| wR(F2) (all data) | 0.108* | 0.120** |

*$w = 1/[\sigma^2(F^2) + (0.04P)^2 + 5.0^2P]$ , where $P = (max(Fo^2,0) + 2Fc^2)/3$
**$w = 1/[\sigma^2(F2) + (0.03P)2 + 9.61P]$ , where $P = (max(Fo^2,0) + 2Fc^2)/3$

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (1)$$

where [DNA] is the concentration of CT-DNA in base pairs, the apparent absorption coefficients $\varepsilon_a$, $\varepsilon_f$ and $\varepsilon_b$ correspond to $A_{obs}$/[DNA], the absorbance for the free-Cu(II) complex (unbound), and the absorbance for the fully-bound complex, respectively. A plot of $[DNA]/(\varepsilon_a–\varepsilon_f)$ versus [DNA] gave a slope $1/(\varepsilon_b–\varepsilon_f)$ and a intercept $1/K_b(\varepsilon_b–\varepsilon_f)$, so the value of $K_b$ can be determined from the ratio of the slope to the intercept.

## 2. 4. DNA Cleavage

Cleavage of plasmid DNA was monitored using agarose gel electrophoresis. Supercoiled pBR322 DNA (0.1 mg/ml, 1.5 μL) in Tris-HCl buffer (5.0 mM, pH 7.2) with 50 mM NaCl was treated with the copper(II) complexes (100–800 μM) in the presence and absence of additives. The concentration of the complexes in DMF or the additives in the buffer corresponded to the quantity after the dilution of the complex stock to the 20 μL final volume using the Tris–HCl buffer. The oxidative DNA cleavage was studied in the presence of $H_2O_2$ (3 mM, oxidizing agent), DMSO (1.5 mM, hydroxyl scavenger), and $NaN_3$ (1 mM, singlet oxygen scavenger). The samples were incubated for 2 h at 37 °C. The loading buffer (4 μL, 12.5% bromophenol blue and 25% xylene cyanol) was subsequently added. The agarose gel (0.8%) containing 2 μL (10 mg/mL stock) of ethidium bromide (EB) was prepared, and the electrophoresis of the DNA cleavage products was performed on it. The gel was run at 60 V for 3 h in TAE (Tris–acetate–EDTA) buffer, and the bands were identified by placing the stained gel under an illuminated UV lamp.

## 2. 5. X-ray Crystallography

Diffraction images were measured at 200 K on a Nonius Kappa CCD diffractometer using Mo $K\alpha$, graphite monochromator ($\lambda$ = 0.71073 Å). Data was extracted using the *DENZO/SCALEPACK* package.[18] The structures were solved by direct methods with the SIR92 and refined on $F^2$ by full matrix last-squares techniques using the CRYSTALS program package, respectively.[19,20] Atomic coordinates, bond lengths and angles, and displacement parameters were deposited at the Cambridge Crystallographic Data Centre. Crystallographic details for the two crystals **1** and **2** are summarized in Table 1.

## 2. 6. Syntheses

The synthesis of 1-benzoyl-3,5-dimethyl-5-(1-benzoylhydrazido) pyrazoline (bzpyzn) was prepared following a published procedure.[21] Briefly, bzpyzn ligand was obtained by the condensation reaction between 1 equivalent of acetylacetone and 2 equivalents of benzohydrazide in methanol by heating the mixture under reflux.

### 2. 6. 1. Syntheses of Tetranuclear Copper(II) Cluster $[Cu_4Cl_6O(C_5H_8N_2)_4]$, 1

Acetylacetone (1.05 mL, 10 mmol) was added to a methanol solution (25 mL) of benzohydrazide (1.36 g, 10 mmol), and the mixture was heated to reflux for 5 h. A solution of $CuCl_2 \cdot 2H_2O$ (1.70 g, 10 mmol) in methanol was added to the above-mentioned bright yellow solution. The green solution was stirred at room temperature for 2 h. After two days, green-brown precipitate was obtained upon the slow evaporation of the solvents at room temperature. The precipitate was recrystallized from acetone/2-propanol/toluene (2:1:1 v/v). Brown-green rod-shaped crystals appeared upon the slow evaporation of the solvents and were washed with ethanol and dried in air. Yield 1.38 g (40%). Anal. Calcd. for $C_{20}H_{32}Cl_6Cu_4N_8O$ (%): C, 27.69; H, 3.72; N, 12.92. Found: C, 27.57; H, 4.01; N, 12.52. IR (KBr, $cm^{-1}$): υC=N = 1572, υN–H = 3335. Electronic spectra in methanol: $\lambda_{max}$(nm), (log ε): 813 (2.24), 311 (3.59).

### 2. 6. 2. Syntheses of Copper(II) Complex, 2

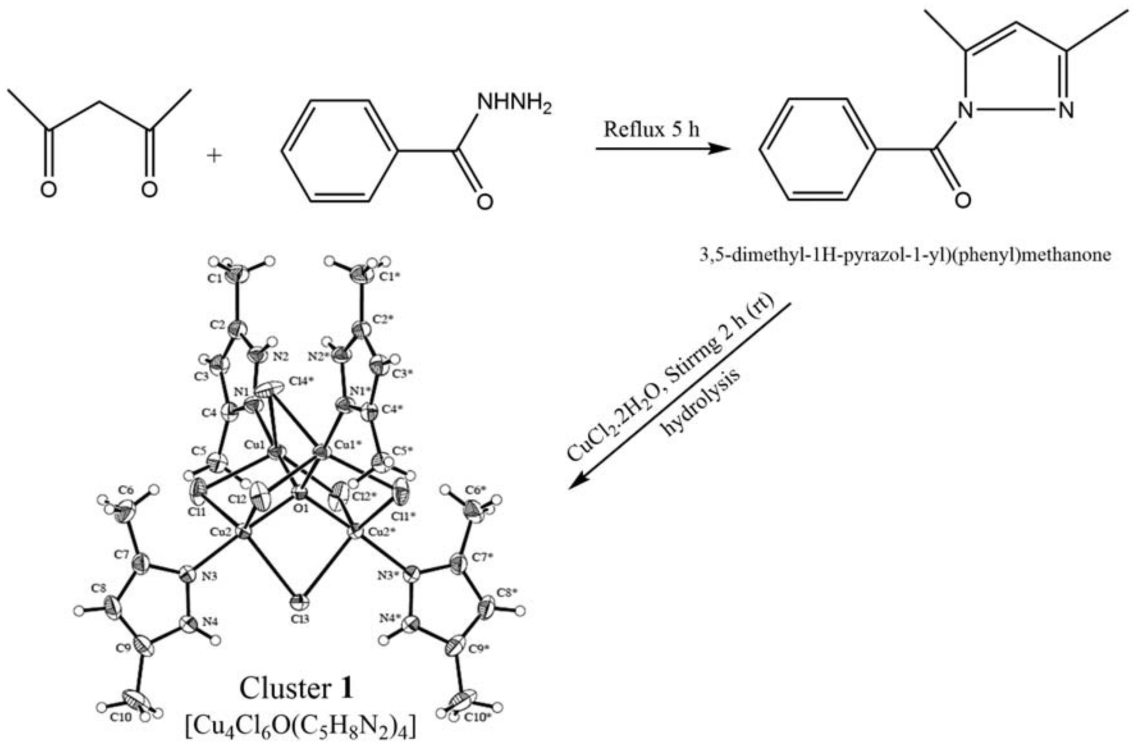
To a methanol solution, (30 ml) of bzpyzn (1.35 g, 4 mmol), $CuCl_2 \cdot 2H_2O$ (0.68 g, 4 mmol) was added, and the green solution was stirred at room temperature for 2 h and left in air at room temperature for slow evaporation. After two days, the initial green color of the solution slowly became brown, and a brown-green crystalline solid separated. The complex was collected by filtration and dried in air. The solid was recrystallized from the DMF solution, and the crystal appeared in about 4–5 days. Yield 0.33 g (20%). Anal. Calcd. for $C_{19}H_{16}CuN_4O_3$ (%): C, 55.40; H, 3.92; N, 13.60. Found: C, 55.89; H, 4.03; N, 13.41. IR (KBr, $cm^{-1}$): υC=O = 1639, υC=N = 1587. Electronic spectra in methanol: $\lambda_{max}$(nm), (log ε): 587(2.21), 442(3.86), 344(4.65), 295 (4.95).

# 3. Result and Discussion

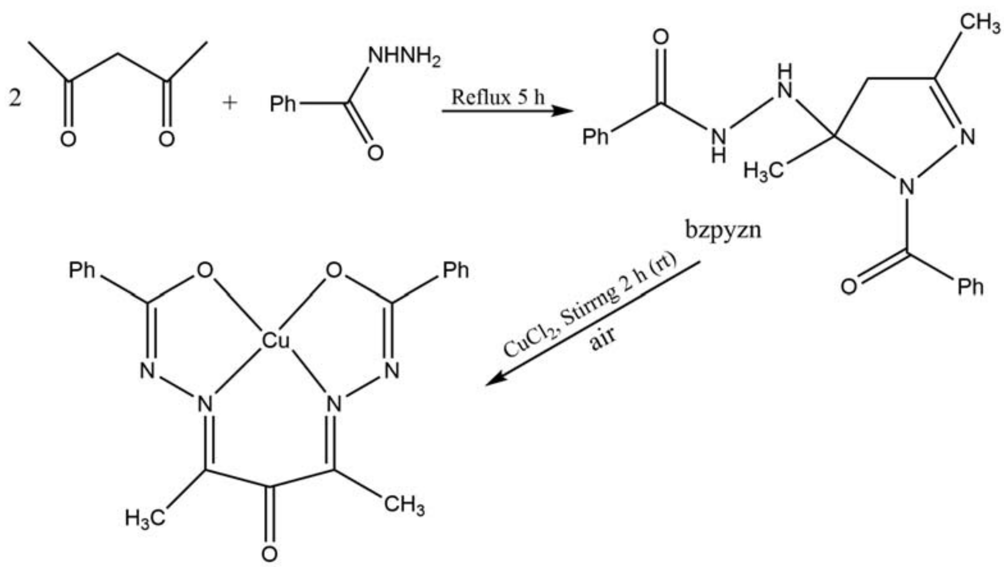
## 3. 1. Synthesis

The complex 1 was synthesized by a two-steps-one-pot reaction with the initial formation of the pyrazol (without its isolation) and then adding the methanolic solution of $CuCl_2$. The pyrazol was prepared in situ from the reactions between acetylacetone and benzohydrazide in methanol under reflux. Initially, (3,5-dimethyl-1H-pyrazol-1-yl)(phenyl)methanone (benzpyrazol) was obtained by the reaction of equimolar amount of acetylacetone and benzohydrazide.[22] The resulting solution which was refluxed for 5 h, was used for the synthesis of the complex without further purification. From the reaction between $CuCl_2$ and benzpyrazol at room temperature followed by its hydrolysis, the brown-green rod-shaped crystalline of the copper(II) cluster **1** was obtained (Scheme 1). The reaction between the pyrazol and $CuCl_2$ at room temperature produced the brown-green rod-shaped crystalline of the copper(II) cluster **1** (Scheme 1). Our search revealed that the synthesis of the cluster was reported by Jaćimović et al. in 2007.[23] They synthesized the green crystals of the copper(II) cluster from the one-pot reaction of 3,5-dimethylpyrazole-1-carboxamide and $CuCl_2$ in a hot ethanol solvent.

Reaction between acetylacetone and benzohydrazide in methanol under reflux will not lead to the formation of the desired acetylacetone bis(benzoylhydrazone) ligand. However, as it is known, this type of the reaction leads to cyclized 3,5-substituted pyrazolines (bzpyzn) (Scheme 2).[21,24]




**Scheme 1** Synthesis of the cluster **1**




**Scheme 2** Synthesis of the complex **2**

Complex **2** was synthesized by the reaction of bzpyzn and $CuCl_2 \cdot 2H_2O$ in methanol under aerobic conditions leads to the ring opening of bzpyzn and complexation of the copper by tetradentate ligand (Scheme 2). After two days, the initial green color of the solution slowly changed to brown, and a brown-green crystalline substance separated. The IR spectrum of bzpyzn showed bands about 3277, 1666 and 1631 $cm^{-1}$ attributable to N–H, C=O and C=N groups, respectively. The IR spectrum of the copper complex **2** did not display any band due to the N–H group. The absence of an N–H group was consistent with the ring opening of bzpyzn and complexation of the copper by tetradentate ligand.[21,24] The middle C-atom of the acetylacetone residue in tetradentate ligand of complex **2** underwent a four-electron-two-proton oxidation in the presence of both water and oxygen and C=O group formed.[24] In complex **2,** the bands observed at 1639 and 1587 $cm^{-1}$ could be assigned to the C=O and C=N groups.[25] In the electronic spectrum, **2** showed a broad absorption band centered at 587 nm because of the spin-allowed d–d transition of the copper(II) ion.[26, 27]

## 3. 2. Crystal Structures

Because a similar structure has been previously reported,[23] the structure and bond lengths and angles of cluster **1** have been submitted as supplementary information.

### 3. 2. 1. Description of Complex 2

Single crystals of complex **2** suitable for X-ray were obtained from the solution of complex **2** in DMF. The complex crystallized in the orthorhombic space group Pbca. The molecular structure of the complex with selected atoms labeled is shown in Figure 1, and selected bond lengths and angles are listed in Table 2. In complex **2**, bzpyzn acted as a tetradentate ligand which bound the metal ion via two amide-O atoms and two imine-N atoms providing an $N_2O_2$ square-plane around the copper(II) ion. The ligand formed two five-membered and one six-membered chelate rings (5-6-5) with a Cu(II) metal center. The copper(II) ion was square planar with a slight tetrahedral distortion. The deviation of the copper(II) center from the $N_2O_2$ square plane is 0.048 Å. The dihedral angles between the two planes [N(2)–Cu(1)–O(1) and N(3)–Cu(1)–O(2)] were 8.32 Å, compared with 0 for a perfectly square-planar arrangement and 90 for a perfect tetrahedral arrangement. The angles at Cu(II) between two donor atoms in the cis position were in the range of 82.19(17) – 95.43(19)° and between two donor atoms in the trans position were in the range of 171.86(18)° and 177.03(17)° (Table 2).

The structure showed that the middle C-atom of the acetylacetone residue had been oxidized with the formation of a C=O group (Figure 1). The C=O bond length in 2 was 1.224(6) Å, which distance is in good agreement with the analogous compounds observed in the literature.[21,24,28] Two Cu–N(imine) bond distances (1.923(4) and 1.930(4) Å) were very similar. The same was also true for the two Cu–O bond distances (1.925(4) and 1.915(4) Å). The distances were comparable with distances observed for square-planar copper(II) complexes having imine-N or deprotonated amide-O coordinating atoms.[26–29]

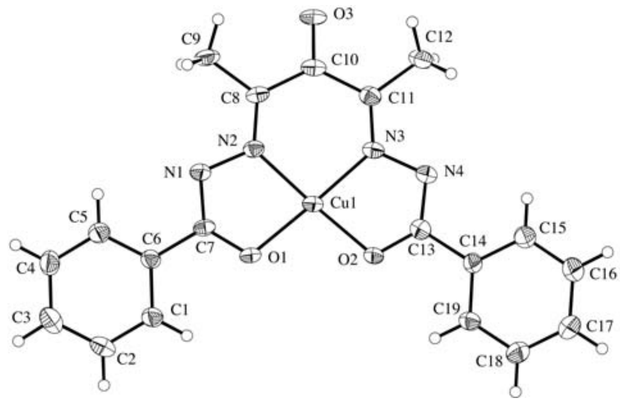



**Fig. 1** The molecular structure of copper(II) complex **2** with labeling of selected atoms

**Table 2.** Selected bond lengths (Å) and angles (°) in complex **2**

| | | | |
|---|---|---|---|
| Cu1–O1 | 1.925 (4) | O1– Cu1–O2 | 100.13 (15) |
| Cu1–O2 | 1.915 (4) | O1– Cu1–N2 | 82.52 (17) |
| Cu1–N2 | 1.923 (4) | O2– Cu1–N2 | 171.86 (18) |
| Cu1–N3 | 1.930 (6) | O1– Cu1–N3 | 177.03 (17) |
| O1–C7 | 1.283 (6) | O2– Cu1–N3 | 82.19 (17) |
| O2–C13 | 1.277 (6) | N2– Cu1–N3 | 95.43 (19) |
| O3–C10 | 1.224 (6) | N2– N1–C7 | 108.80 (5) |
| N1–N2 | 1.391 (6) | N1– N2–Cu1 | 113.50 (3) |
| N1–C7 | 1.331 (6) | C8– C10–O3 | 116.30 (5) |
| N2–C8 | 1.301 (6) | C8– C10–C11 | 126.80 (4) |
| N3–C11 | 1.302 (6) | | |

## 3. 3. DNA Binding Studies

### 3. 3. 1. Absorption Spectral Studies of CT-DNA with 1

UV–Visible spectroscopy was performed to study the interaction of the complexes with DNA keeping the complex concentration constant (40 μM) and varying the concentration of CT-DNA (0–60 μM). The change in absorbance values at 260 nm was used to evaluate the intrinsic binding constant $K_b$. The absorption spectra of 1 in the presence of CT-DNA are shown in Figure 2. With increasing the concentrations of CT-DNA, the complex exhibited hyperchromism without a shift in band position at 260 nm.

The hyperchromism indicated a strong interaction between the complexes and CT-DNA mainly through groove binding.[30,31]

The use of a tetranuclear copper(II) cluster with large steric hindrance led to the poor binding affinity interaction of the complex with the double stranded CT-DNA. Since the complex cannot be intercalated well with DNA, classical intercalative interaction was excluded. However, since DNA possesses several hydrogen bonding sites which are accessible both in the minor and major grooves, a favorable hydrogen bonding may be formed between the amine –NH– groups of the pyrazol ligand in cluster with the base pairs in CT-DNA.[32,33]

Analysis of the spectrum data using Equation 1 in the presence of DNA (Figure 2) gave a binding constant, $K_b$, of $0.45 \times 10^4$ $M^{-1}$ (r = 0.967 for seven points) for the copper cluster. From the binding constant value, it was clear that the cluster had moderate interaction with CT-DNA.

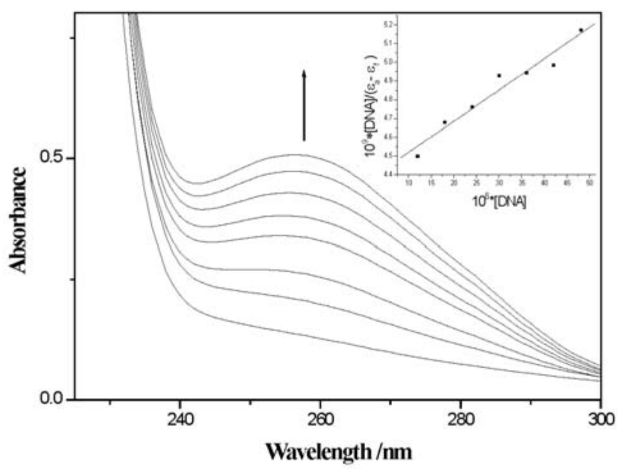



**Fig. 2** Absorption spectra of complex **1** (40 μM) in Tris–HCl buffer (pH = 7.2) with the increase in molar ratio of DNA to complex (0–60 μM). Arrow shows the absorbance changing upon the increase of DNA concentration. The inset shows plot of [DNA]/($\varepsilon_a$–$\varepsilon_f$) vs. [DNA] for titration of CT–DNA with the complex

## 3. 3. 2. Absorption Spectral Studies of CT-DNA with 2

Figure 3 illustrates the representative UV–Vis spectra for the binding of complex **2** with DNA at a constant complex concentration 70 μM and varying the concentration of CT-DNA (0–60 μM). Increasing the DNA concentration showed hypochromicity in the absorption spectra. The change in hyperchromic to hypochromic shift from the copper(II) cluster **1** (Figure 3) to complex **2** indicated that the type of binding mode was different. This can be attributed to a difference in the structure of the two complexes. The observable hypochromism is usually characterized by the non-covalently intercalative binding of the compound to the DNA helix.[31,34,35] Complex **2** bound to DNA through intercalation and resulted in hypochromism due to the strong stacking interaction between the aromatic chromophore of the complex and the base pairs of DNA.[35]

The intrinsic binding constants $K_b$ for complex **2** were determined by monitoring the change of the absorption

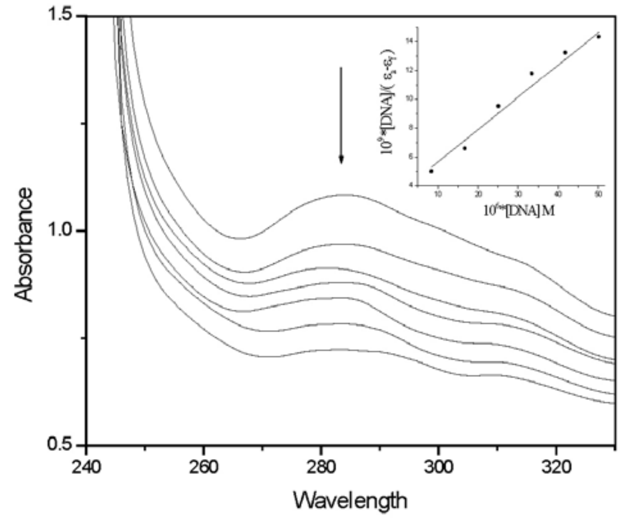



**Fig. 3** Absorption spectra of complex **2** (70 μM) in Tris–HCl buffer (pH = 7.2) with the increase in molar ratio of DNA to complex (0–60 μM). Arrow shows the absorbance changing upon the increase of DNA concentration. The inset shows plot of [DNA]/($\varepsilon_a$–$\varepsilon_f$) vs. [DNA] for titration of CT–DNA with the complex

intensity of the charge transfer spectral band of the complex by increasing the concentration of CT-DNA (Figure 3). The binding constant ($K_b$) for **2** is $2.39 \times 10^4$ $M^{-1}$ (r = 0.986 for six points), suggests that a strong interaction exists between complex **2** and CT-DNA due to its square planar nature.[36,37] The binding strength of the complex **2** is notably larger than the free ligand, suggesting that the electronic configuration of the metal center may be an important factor affecting the DNA binding affinities of such as compound.

The intrinsic binding constants $K_b$ obtained for **1** and **2** were lower than those observed for the typical classical intercalator ethidium bromide (EB), $K_b = 1.23 \times 10^6$ $M^{-1}$.[38] Therefore, interaction of the two complexes with CT-DNA is considered to be weaker than with classical intercalator. However, the $K_b$ value for the complex **2** is higher than some reported mononuclear copper(II) complexes, such as [Cu(bpy)(Gly)Cl] · $2H_2O$, $1.84 \times 10^3$ $M^{-1}$, [Cu(dpa)(Gly)Cl] $2H_2O$, $3.10 \times 10^3$ $M^{-1}$,[1] [Cu(L)(bpy)Cl] (HL = (E)-3-(2-hydroxyphenylimino)-N-*o*-tolylbutanamide), $1.55 \times 10^3$ $M^{-1}$,[18] and is comparable to that observed for [CuL1]$(ClO_4)_2$ (L1 = *N,N'*-bis-pyridin-2-ylmethyl-butane-1,4-diimine), $2.6 \times 10^4$ $M^{-1}$,[4] $[Cu(nfH)_2]Cl_2$ (nfH = norfloxacin), $4.08 \times 10^4$ $M^{-1}$,[10] and [Cu(*o*-vanile)(phen)], $2.13 \times 10^4$ $M^{-1}$.[37]

## 3. 4. Supercoiled pBR322 Plasmid DNA Cleavage Studies

Gel electrophoresis experiments using pBR322 circular plasmid DNA were performed with complexes **1** and **2** in the presence and absence of $H_2O_2$ as an oxidant. At micro-molar concentrations, for 3 h incubation periods, in the absence and presence of an oxidizing agent, complex 2 exhibited no nuclease activity. However, even in the absence of an oxidant, complex **1** exhibited significant DNA cleavage activity (Figure 4, lane 2). Control experiments using DNA alone resulted in no significant cleavage of pBR322 circular plasmid DNA, even after longer exposure times (Figure 4, lane 1). From the observed results, it was concluded that complex 1 effectively cleaved the DNA as compared to control DNA. The copper cluster at a higher concentration (800 μM) showed more cleavage activity than at the lower concentration (100 μM) of cluster 1 (Figure 4, lanes 3–5). This shows that the concentration of optimal value led to extensive degradations, resulting in the conversion of the supercoiled form (Form-I) into an open-circular form (Form-II).

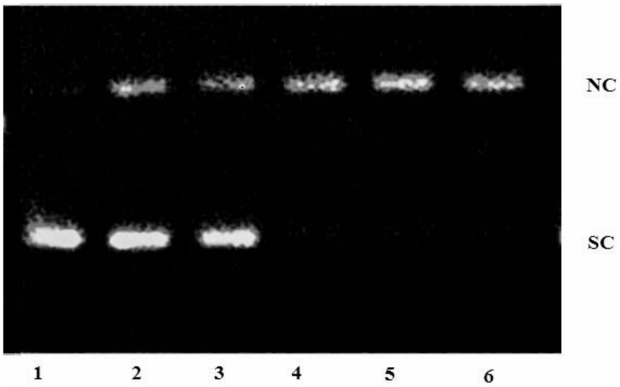



**Fig. 4** Agarose gel electrophoresis diagram showing the chemical nuclease activity of the complexes **1** using Supercoiled pBR322 plasmid DNA (0.1 mg/ml, 1.5 μL): lane 1, DNA control; lane 2, DNA + 1 (100 μM); lane 3, DNA + 1 (500 μM); lane 4, DNA + 1 (800 μM); lane 5, DNA + 1 (800 μM) + $NaN_3$ (1 mM); lane 6, DNA + 1 (800 μM) + DMSO (1.4 mM)

The nuclease activity of the complexes was also investigated in the presence of a free radical scavenger, dimethylsulfoxide (DMSO), and a singlet oxygen quencher, azide ion ($NaN_3$). The presence of radical scavengers (DMSO) and a singlet oxygen quencher ($NaN_3$) did not significantly reduce the efficiency of DNA cleavage (Figure 4, lanes 6, 7), ruling out the possibility of the involvement of diffusible hydroxyl radicals and a singlet oxygen in the cleavage.[8,39]

The copper(II) cluster may be capable of bringing about hydrolytic cleavage of DNA. This is not surprising since hydrolytic cleavage requires the coordinative binding of the copper(II) cluster to either DNA bases or phosphate.[40] Since complex 1 can interact by hydrogen bonding with the base pairs in DNA, one can expect it to promote hydrolytic cleavage.

## 4. Conclusion

The present work describes the synthesis, characterization, and DNA binding and cleavage studies of copper(II) compounds 1 and 2. Single crystal X-ray diffraction studies revealed that the two complexes have two different structures, namely a tetranuclear cluster and a mononuclear square-planar. The binding of the complexes with CT-DNA was studied by UV-Vis spectroscopy, and their strong binding ability was revealed. Complex 2 exhibited no nuclease activity; however, complex 1 exhibited significant DNA cleavage activity.

## 5. Supplementary material

The deposition numbers of the studied complexes, **1** and **2** are CCDC 963061 and 963060, respectively. These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data-request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033.

## 6. Acknowledgments

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## Povzetek

Dva bakrova(II) kompleksa, klaster **1** in enojederni **2**, sta bila sintetizirana pri reakciji acetilacetona in benzohidrazida (v razmerju 1:1 za **1** in 1:2 za **2**) z $CuCl_2$ v metanolu. Pri **2**, ki je nov kompleks, je ligand tetradentatni in vezan na kovinski ion preko dveh amidnih O atomov in dveh iminskih N atomov in tvori $N_2O_2$ kvadratno-planarno geometrijo okoli bakrovega(II) ion. Podatki dobljeni iz absorpcijskih spektrov kažejo, da obe bakrovi(II) spojini lahko interagirata s CT-DNA ($K_b = 0.45 \times 10^4$ $M^{-1}$ za **1** in $K_b = 2.39 \times 10^4$ $M^{-1}$ za **2**). Na super coiled plasmidu pBR322 je bila testirana zmožnost **1** in **2** cepiti DNA v prisotnosti ali odsotnosti $H_2O_2$ kot oksidanta. V obeh primerih, tako v prisotnosti kot odsotnosti oksidanta, kompleks **2** nima nukleazne aktivnosti, medtem ko tudi v odsotnosti oksidanta kompleks **1** izkazuje veliko aktivnost cepitve DNA.

## Supplement Material

# Synthesis, Structure Characterization, DNA Binding, and Cleavage Properties of Mononuclear and Tetranuclear Cluster of Copper(II) Complexes

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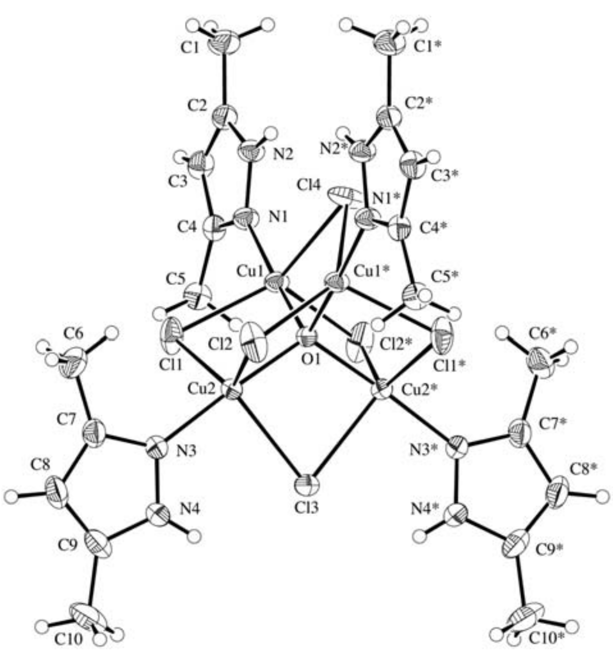


**Fig. S** The molecular structure of tetranuclear cluster of copper(II) complex with labeling of selected atoms

**Table S.** Selected bond lengths (Å) and angles (°) in complex **1**[a]

| **Complex 1** | | | |
|---|---|---|---|
| Cu1–Cl1 | 2.3580 (11) | Cl1– Cu1–Cl2* | 116.83 (5) |
| Cu1–Cl4 | 2.4386 (19) | Cl1– Cu1–O1 | 85.46 (4) |
| Cu1–Cl2* | 2.4069 (12) | Cl4– Cu1–O1 | 82.59 (8) |
| Cu1–N1 | 1.9620 (3) | Cl1– Cu1–N1 | 96.12 (9) |
| Cu1–O1 | 1.9072 (17) | Cl4– Cu1–N1 | 92.75 (10) |
| Cu2–Cl1 | 2.3567 (10) | O1– Cu1–N1 | 174.33 (11) |
| Cu2–Cl2 | 2.3810 (10) | Cl1– Cu2–Cl2 | 125.74 (5) |
| Cu2–Cl3 | 2.4901 (11) | Cl1– Cu2–Cl3 | 117.96 (4) |
| Cu2–N3 | 1.9530 (3) | O1– Cu2–N3 | 175.97 (5) |
| Cu2–O1 | 1.9091 (16) | Cu1– O1–Cu2 | 107.53 (17) |

[a] symmetry codes:–x+3/2, y, –z+3/2.