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Research Paper

# Synthesis, DNA Binding and Activity of Mixed Ligand Copper(II) Complexes with Sulfur Containing Ligand: 1-Hydroxy-2-Acetonaphthonetosylhydrazone

Chemistry

**KEYWORDS :** Copper(II) complexes, 1-hydroxy-2-acetonaphthonetosylhydrazone, pUC19 DNA, DNA Binding and Nuclease activity.

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## ABSTRACT

*Four new mixed ligand copper(II) complexes of the composition, [Cu(ohtant)(phen)Cl] (1) [Cu(ohtant)(bpy)Cl] (2)[Cu(ohtant)(phen)]$ClO_4$ (3) [Cu(ohtant)(bpy)]$ClO_4$ (4) (where ohtant = 1-hydroxy-2-acetonaphthonetosylhydrazone, phen = 1,10-phenanthroline and bpy = 2,2'-bipyridine) have been synthesized. These complexes are characterized on the basis of molar conductance, IR and UV spectral studies. The DNA cleavage activity of all the copper(II) complexes was carried out on double-stranded supercoiled pUC19 DNA by using gel electrophoresis experiment in the presence of ascorbic acid as a reducing agent. The binding of CT-DNA with copper(II) complexes was investigated by electronic absorption spectroscopy. The binding constant value of $4.9x10^5M^{-1}$ for complex (1) with CT-DNA suggests good intercalative binding mode of the complex. The results confirm that the mixed ligand complexes 2,3 and 4 showed good nuclease activity.*

## 1. Introduction

The interaction of transition metal ions with biological molecules gains paramount importance in recent years particularly in the field of medicine. The metal ions are also known to accelerate drug action. The interaction of metal ions with nucleic acids and nucleic acid constituents has been studied in recent years 1, 2, 3. Transition metal complexes have been widely used for binding and cleaving double stranded DNA under physiological conditions because of their unique spectral and electrochemical properties and due to the fact that by changing the ligand environment 4, 5. These metal complexes are of current interest due to their various applications in nucleic acid chemistry like sequence-specific binding agents, foot-printing and for modeling the restriction of enzymes in gene research and used as new structural probes for the therapeutic agents in cancer therapy 6, 7, 8, 9, 10, 11, 12, 13, 14. The development of modern medicinal inorganic chemistry, stimulated by the serendipitous discovery of cis-platin has been facilitated by the inorganic chemists. Platinum(II) complexes are used as anticancer drugs since long and among them cis-platin has proven to be a highly effective chemotherapeutic agent for treating various types of cancers like ovarian, testicular, head and neck cancer 15, 16, 17. But its use is limited because it has many side effects such as hair follicle, neuro toxicity and the lining of gastro-intestinal tract due to drug resistance phenomenon. To overcome these side effects and limited activity of cis-platin, great efforts have been made to synthesize other alternatives such as transition metal complexes of Cu(II) which act as better antitumor drugs 18, 19, 20, 21. The copper(II) complexes have found possible medical uses in treatment of many diseases because of their biologically accessible redox potential and high nucleobase affinity and are used as potential agents for the cleavage of DNA. The ability of copper(II) complexes to cleave DNA upon photoactivation under physiological condition have also received a great attention 22, 23, 24. The first copper(II) complexes viz. tris copper complexes bis(phen)copper complexes are used as chemical nuclease that cleaves DNA in the presence of $H_2O_2$ and thiol reported by Sigman and coworkers. Shanta Dhar and coworkers recently reported the sulfur containing ligand in copper(II) complexes act as photosensitizer in visible light induced DNA cleavage reactions on visible light irradiation 25, 26, 27, 28, 29. The sulfur to copper charge transfer band and copper d-d band excitation in the ternary structures having Cu-S bond significantly augments the DNA cleavage activity via a mechanistic pathway involving formation of singlet oxygen that cleaves DNA 30.

A survey of literature reveals that copper(II) complexes of certain ligands containing sulfur and heterocyclic moiety show better nuclease activity 31, 32, 33, 34, 35. The present work stems from the interest to design copper(II) complexes suitable for Photo dynamic therapy (cancer treatment) by using sulfur containing ligand as photo sensitizers and planar chelating heterocyclic bases as groove binders to DNA.

## 2. Experimental

### 2.1 Reagents and materials

All the reagents and chemicals were purchased from commercial sources and used without further purification. Solvents used for the preparation of ligand and its copper(II) complexes, for electrochemical and spectroscopic measurements were purified by standard procedures 36. The calf-thymus DNA and double stranded supercoiled pUC19 DNA were purchased from Banglore Geni (India), Agarose (Molecular biology Grade) and ethidium bromide were from Sigma(USA). Tris-HCl/ NaCl buffer solution was prepared using Millipore water.

### 2.2 Physical measurements

Molar conductance of the copper(II) complexes were measured using digital conductivity meter of cell constant (1.00). Approximately $1x10^{-3}$M solution of the complexes in DMF was employed for the conductance measurements. The IR spectra were recorded on SHIMADZU FTIR-8400S spectrophotometer using KBr pellets in the range of 4000- 400$cm^{-1}$. Electronic absorption spectra were recorded on a JASCO V-550 UV-Visible spectrophotometer using DMF as solvent in the range of 200-800nm.

### 2.3 Synthesis of 1-Hydroxy-2-acetonaphthonetosylhydrazone

1-Hydroxy-2-acetonaphthone (10g) in 250ml of ethanol was treated with tosyl hydrazine (10g) in 250ml of ethanol. This mixture was refluxed for 6 hours and was allowed to stand overnight. It was filtered and to the filtrate added water and ice cubes. The separated 1-Hydroxy-2-acetonaphthonetosylhydrazone was suction filtered, washed with water, dried and recrystallised from ethanol. **Yield = 60%**.

### 2.4 Synthesis of [Cu(ohtant)(phen)Cl]

To a solution of 1-Hydroxy-2-acetonaphthonetosylhydrazone (3.54g, 10mM), added triethyl amine (1.01g) and 1,10-phen-

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anthroline (1.98g, 10mM) in ethanol (100ml). To this ligand mixture added a solution of copper chloride (1.70g, 10mM) in ethanol (100ml). The complex settled was suction filtered, washed with ethanol, dried in air and then in vacuo over anhydrous calcium chloride. **Yield = 68%**.

**2.5 Synthesis of [Cu(ohtant)(bpy)Cl]**
To ethanolic solution of 1-Hydroxy- 2-acetonaphthonetosylhydrazone (3.54g, 10mM), added triethyl amine (1.01g) and ethanolic solution 2,2'-bipyridine (1.56g, 10mM). To this ligand mixture added ethanolic solution of copper chloride (1.70g, 10mM). The complex settled was suction filtered, washed with ethanol, dried in air and then in vacuo over anhydrous calcium chloride. **Yield=73%**

**2.6 Synthesis of [Cu(ohtant)(phen)]$ClO_4$**
To ethanolic solution of 1-Hydroxy- 2-acetonaphthonetosylhydrazone (3.54g, 10mM), added triethyl amine (1.01g) and ethanolic solution of 1,10-phenanthroline (1.98g, 10mM). To this ligand mixture added ethanolic solution of copper perchlorate (3.70g, 10mM). The complex settled was suction filtered, washed with ethanol, dried in air and then in vacuo over anhydrous calcium chloride. **Yield = 71%.**.

**2.7 Synthesis of [Cu(ohtant)(bpy)]$ClO_4$**
To ethanolic solution of 1-Hydroxy-2-acetonaphthonetosylhydrazone (3.54g, 10mM), added triethyl amine (1.01g) and ethanolic solution 2,2'-bipyridine (1.56g, 10mM). To this ligand mixture added ethanolic solution of copper perchlorate (3.70g, 10mM). The complex settled was suction filtered, washed with ethanol, dried in air and then in vacuo over anhydrous calcium chloride.

**Yield = 73%.**

**2.8 DNA Binding**
**2.8.1 Preparation of stock DNA solution**
Concentrated CT – DNA stock solution was prepared in 50mM Tris- HCl/ 50mM NaCl in water at pH 7.2 and the concentration of DNA solution was determined by UV- absorbance at 260nm. The molar absorption coefficient was taken as 6600 $cm^{-1}$ $M^{-1}$. The solution of CT- DNA in 50mM Tris- HCl/ 50mM NaCl in water at pH 7.2 gave a ratio of UV absorption at 260nm and 280nm $A_{260}/A_{280}$ of. Ca 1.8-1.9, indicating that the DNA was sufficiently free of protein. All stock solutions were stored at $4^0$C and were used within four days 37.

**2.8.2 Absorption spectra**
Absorption spectra for the DNA binding studies were recorded on Thermo Scientific Helious alpha UV-visible spectrophotometer using 1cm quartz micro cuvettes. Electronic spectra of the complexes were recorded by keeping the concentration of the complex constant while varying the concentration of the nucleic acid before and after addition of CT DNA ($1x10^{-5}$M, $2x10^{-5}$M, $3x10^{-5}$M, $4x10^{-5}$M) in the 5mM Tris- HCl buffer at pH 7. For the complexes, the binding constants ($K_b$) have been determined from the spectroscopic titration data using the following equation.

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

The apparent extinction coefficient ($\boldsymbol{\varepsilon}_a$) was obtained by calculating $A_{obsd}$ / [Cu]. The terms $\boldsymbol{\varepsilon}_f$ and $\varepsilon_b$ corresponds to the extinction coefficient of free (unbound) and the fully bound complexes respectively. A plot of [DNA] / ($\boldsymbol{\varepsilon}_a - \varepsilon_f$) versus [DNA] will give a slope $1/(\boldsymbol{\varepsilon}_b - \varepsilon_f)$ and an intercept $1/ K_b (\boldsymbol{\varepsilon}_b - \varepsilon_f)$. $K_b$ is the ratio of the slope to the intercept 38.

**2.9 Nuclease activity**
The cleavage of pUC19 DNA was monitored by agarose gel electrophoresis. The electrophoresis experiments were performed with sample containing 1.5µl of pUC 19 DNA (250ng), 5µl (30µM) of copper complex, 4µl (100µM) of ascorbic acid and diluted to 20µl with 50mM Tris-HCl buffer (pH 7.2). The samples were incubated for 1 hr at $37^0$C. After incubation, to the samples added loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol and electrophoresis was carried out for 2 hours at 50 V on 0.7% agarose gel containing 1.0µg/ml of ethidium bromide. After electrophoresis the bands were observed under Spectroline Ultraviolet transilluminator and photographed to determine the extent of DNA cleavage 39.

**3. Results and Discussion**
The ligand form octahedral complexes 1, 2, 3, 4 with $CuCl_2.2H_2O$/ copper perchlorate solution in ethanol. The proposed structure for the complexes are shown in figure 1. All the four complexes were colored, stable in air and atmosphere. Complexes 1 and 2 are dark brown colored and complexes 3 and 4 were dark green colored. These complexes were insoluble in water, moderately soluble in acetonitrile and chloroform and completely soluble in DMF. The molar conductance values of complex 1 and 2 were too low to account for any dissociation of the complexes in DMF, indicating the non-electrolytic nature of the complexes in DMF and for the complex 3 and 4 showed 1:1 electrolyte properties of the complexes 40, 41.

**3.1 IR Spectral analysis**
The IR spectral analysis for all the complexes did not show a broad peak at 3500$cm^{-1}$ , which indicate that the 'O' is coordinated to the metal. The complexes [Cu(ohtant)(phen)]$ClO_4$ and [Cu(ohtant)(bpy)]$ClO_4$ showed intense peak at 1085,1089,1097 and 1081 $cm^{-1}$, respectively, which corresponded to the ionic perchlorate.

**3.2 UV Spectral analysis**
The electronic spectra for the complexes were recorded in DMF at approximately 0.001M concentration. The mixed ligand complexes exhibited ligand based π- π* transitions in the UV region. All the complexes exhibited a band at 359nm, 369nm and 400nm, that corresponds to CT band. The complexes also exhibited bands at 740nm, 762nm, 625nm, 650nm in the visible region, which may be assigned to $^2B_{1g} \rightarrow {}^2B_{2g}$, $^2$Eg in a $D_{4h}$ environment.

**4.1 DNA Binding activity**
Electronic absorption spectroscopy is universally employed to determine the binding of complexes with DNA. Complex bound to DNA through intercalation usually results in hypochromism and red shift (bathochromism), due to the intercalative mode involving a strong stacking interaction between aromatic chromophore and the base pairs of DNA. The extent of the hypochromism is commonly consistent with the strength of intercalative interaction [42]**.**The intrinsic binding constants, $k_b$ obtained from the ratio of slope to the intercept, from the plots of [DNA] / ($\varepsilon_a - \varepsilon_f$) versus [DNA] were shown in **Table 1.**

| S.No | Complexes | Intrinsic Binding constant, $K_b$ x$10^5$ $M^{-1}$ |
|---|---|---|
| 1 | [Cu(ohtant)(phen)Cl] | 4.9 |
| 2 | [Cu(ohtant)(bpy)Cl] | 1.28 |
| 3 | [Cu(ohtant)(phen)]$ClO_4$ | 3.7 |
| 4 | [Cu(ohtant)(bpy)]$ClO_4$ | 1.9 |

**Table 1: The intrinsic binding constants, $K_b$ of the synthesized mixed ligand copper (II) complexes of 1-hydroxy-2-aceto naphthonetosylhydrazone.**

The binding property of the phen complexes is high in comparison to their bpy analogues. This property could be due to the presence of extended planar aromatic rings facilitating non covalent interaction with the DNA.

**4.2 Nuclease activity**
The cleavage efficiency is measured by determining the ability of the complex to convert the supercoiled DNA (SC) to nicked circular form (NC). When the plasmid DNA is subjected to electrophoresis, fastest migration is observed for supercoiled form (Form I). If one strand is cleaved, the supercoiled will relax to

produce nicked circular form (Form II) which migrates slowly. If both the strands are cleaved, linear conformation (form III) is produced, which migrates between the other two forms [43].

The cleavage of pUC 19 DNA was monitored by agarose gel electrophoresis. The electrophoresis experiments were performed with sample containing 3µl (250ng) of SC pUC19 DNA, 5µl (30µM) of copper complex, 2µl (100µM) of ascorbic acid and diluted to 20µl with 50mM Tris – HCl buffer containing 50mM NaCl (pH 7.2). Gel electrophoresis **figure 2** showing the cleavage of SC pUC19 DNA by the copper complexes in DMF, in buffer containing 50mM Tris-HCl/50mM NaCl (pH 7.2) in the presence of Ascorbic acid at $37^0$C is shown in **figure 2**. From the results we infer that the mixed ligand complexes of phen showed good nuclease activity.

When pUC19 DNA was treated with the complex, [Cu(ohtant)(phen)]$ClO_4$, there was complete conversion of supercoiled form (Form I) form to nicked circular form (Form II). This shows that there is complete cleavage of pUC19 DNA by the complex. Thus the complex exhibits a good nuclease activity as shown in **figure 2.**

When pUC19 DNA treated with the complex [Cu(ohtant)(bpy)]$ClO_4$, was investigated, there was partial conversion of SC (Form I ) of pUC19 DNA to NC form (Form II), as evident from **figure 2** , it shows that this complex exhibit only a partial nuclease activity when compared to complex [Cu(ohtant)(phen)]$ClO_4$, which exhibits complete nuclease activity.

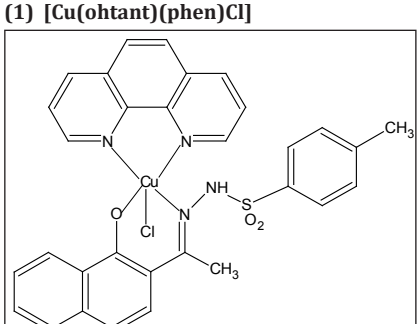
When complex [Cu(ohtant)(phen)Cl] was analysed for DNA cleavage activity, there was no conversion of SC form (Form I) to NC form (Form II) of pUC19 DNA, as shown in figure 2. As the complex fails to cleave SC pUC19 DNA, it exhibits no DNA cleavage property.

When pUC19 DNA was treated with the complex, [Cu(ohtant)(bpy)Cl], there was conversion of almost half of the SC form (Form I ) form to NC form (Form II). **Figure 2.** Thus this complex comparatively showed a good nuclease activity when compared with complex [Cu(ohtant)(phen)Cl].

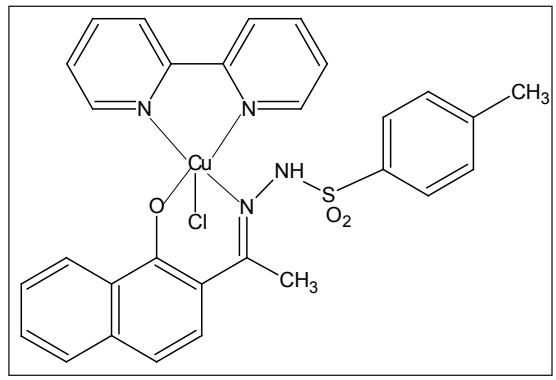
**Conclusion**

In the present study, novel mixed-ligand complexes of copper(II) using 1-Hydroxy -2-acetonaphthonetosylhydrazone and DNA binder such as 1,10-phenanthroline and 2,2'-bipyridine was used for synthesizing all the complexes. The interaction of the synthesized copper(II) complexes with DNA (Calf-thymus CT DNA) was done by performing DNA binding studies using UV-Absorption spectrophotometer. All the four complexes exhibit DNA binding property with Intrinsic Binding constant ($K_b$) between 1.28 X $10^5$ and 4.9 X $10^5$ $M^{-1}$. Highest Intrinsic Binding constant was observed for the complex **(1).** DNA cleavage studies of the complexes were done using pUC19 DNA in the presence of ascorbic acid by performing agarose gel electrophoresis. Though all the complexes exhibit DNA binding property, complex **(1)** failed to show the nuclease activity **(Figure 2)**. Further studies including cytotoxicity assay is needed to confirm whether the complexes used in this study destroy the cancer cells or not.

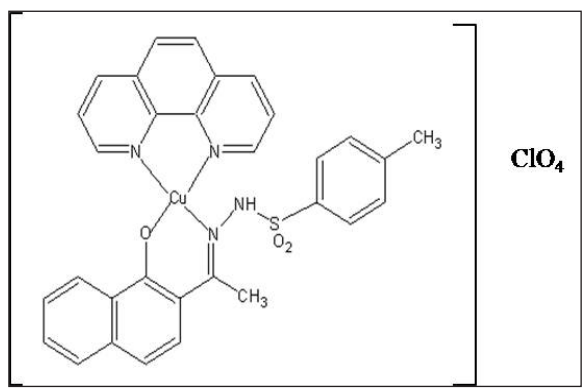
**(1) [Cu(ohtant)(phen)Cl]**




**(2) [Cu(ohtant)(bpy)]Cl**




**(3)[Cu(ohtant)(phen)]ClO4**




**(4)[Cu(ohtant)(bpy)]ClO4**

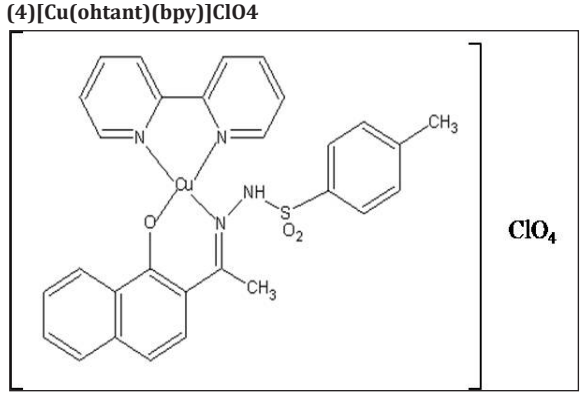



**Figure 1. Proposed Structures for the Complexes.**

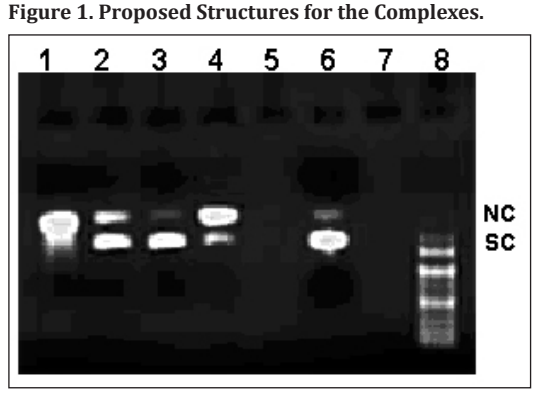



**Figure 2: Illustrates the gel electrophoretic separation showing the cleavage of plasmid pUC19 DNA induced by the four complexes under identical reaction conditions using Ascorbic acid as a reducing agent.**
**Lane 1: DNA + [Cu(ohtant)(phen)]$ClO_4$ + Ascorbic acid;**
**Lane 2: DNA + Cu(ohtant)(bpy)]$ClO_4$ + Ascorbic acid; Lane**

**3: DNA + [Cu(ohtant)(phen)Cl] + Ascorbic acid; Lane 4: DNA +[Cu(ohtant)(bpy)Cl] + Ascorbic acid; Lane 6: DNA control. Lane 8: DNA Ladder.**

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