



## SYNTHESIS, SPECTROSCOPIC CHARACTERIZATION and CATECHOLASE-LIKE ACTIVITY OF NOVEL AMINOKETOOXIME LIGAND and Cu(II) COMPLEX

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### ABSTRACT

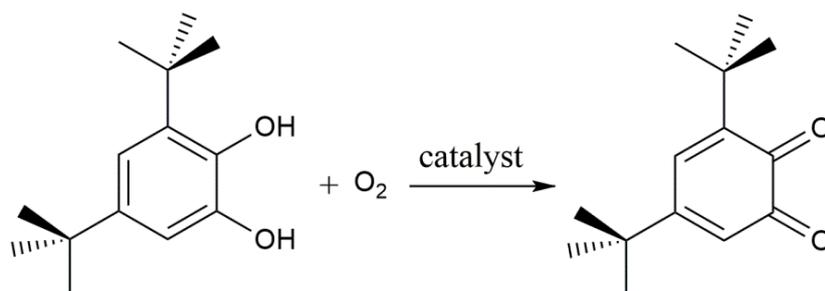
*A new ketooxime ligand, 2-(biphenyl-4-yl)-N'-hydroxy-2-oxo-N-(pyridine-2-yl)acetimidamide (HL), and its mononuclear Cu(II) complex ([CuL<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>]) with a metal:ligand ratio of 1:2 were synthesized. The structures of these compounds were characterized by elemental analyses, ICP-OES, <sup>1</sup>H- and <sup>13</sup>C-NMR, FT-IR spectra, magnetic susceptibility and molar conductivity measurements. According to the stoichiometric and spectroscopic results, the ligand coordinated Cu(II) ion through its oxime oxygen atom and -NH- group bonded to aromatic ring of 2-aminopyridine. The synthesized complex was tested for its catecholase-like activity for the oxidation of 3,5-di-*t*-butylcatechol to 3,5-di-*t*-butylquinone. Catalytically efficiency of the complex and kinetic parameters were also determined.*

**Keywords:** Oxime, complex, enzyme, catecholase, spectroscopy

### INTRODUCTION

Oxime based metal complexes have great deal of importance since their discovery in early 1900s [1]. Today, oximes and oxime based complexes have various application fields from medicine to chemical industry. Some oxime derivatives have physiological and biological activity. Some are widely used in organic, analytical, inorganic, pigment chemistry [2]. Copper is an essential element, a part of many vitamins, hormones, enzymes, and respiratory pigments, and is involved in metabolism, tissue respiration, etc. [3]. Cu(II) containing oxime

complexes show relatively higher biological activity compared to other metal complexes [4]. Metal complexes have also been used extensively for biomimetic studies for their catalytic properties, hence their ability to stabilize unusual oxidation states and possibilities for magnetic interaction between two metal ions [5,6]. Catecholase (catechol oxidase: EC 1.10.3.1) is a member of oxidoreductases which catalyzes the conversion of 3,5-di-*t*-butylcatechol to 3,5-di-*t*-butylquinone [7]. Catecholase-like activity shown by synthetic metal complexes studied by researchers.



**Figure 1.** Oxidation of the 3,5-di-*t*-butylcatechol by the catalyst

## EXPERIMENTAL

### Materials and Methods

All reagents were purchased commercially and used as received. The FT-IR spectra were recorded as KBr pellets on a Shimadzu IRPrestige-21 FT-IR Spectrophotometer. UV-Vis spectra were obtained on a PG T80+ Spectrophotometer. <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of the ligand were recorded with Varian Unity (400 MHz) from CDCl<sub>3</sub> solutions with TMS as internal standart. The stoichiometric analyses (C, H and N) of the ligand and the complex were performed using LECO 932 CHNS analyzer. Metal content was measured on Perkin Elmer Optima 5300 DV ICP-OES Spectrometer in solution prepared by decomposing the respective complex in hot concentrated HNO<sub>3</sub>. Magnetic susceptibility measurements were carried out at room temperature in powder form on a Sherwood Scientific Magnetic Susceptibility Balance (Model MX1). The conductance measurements were carried out using an Optic Ivymen System conductivity meter. Melting point determinations were performed with a digital melting point instrument from Electrothermal model IA 9100.

## Synthesis of Aminoketooxime Ligand (HL)

Chloroketooxime 0.015 mole is dissolved in ethanol and cooled to -5 °C in ice-salt bath. 0.03 mole of 2-amino pyridine-ethanol solution added dropwise. Mixture stays 2 hrs stirring at -5 °C. The precipitation filtered and washed 1% NaHCO<sub>3</sub> and ethanol and left to dry.

Yellow compound; yield: 68%; m.p.: 186 °C. Anal. Calc. for C<sub>19</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>: C, 71.91; H, 4.76; N, 13.24. Found: C, 72.13; H, 4.64; N, 13.36%; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, ppm): 9.81 (s, 1H, O-H), 8.69 (s, 1H, N-H), 7.45-8.08 (m, 13H, Ar-H); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, ppm): 182.13 (carbonyl C), 154.27 (oxime C), 126.74-163.48 (aromatic C); FT-IR (KBr, cm<sup>-1</sup>): 3288 b (O-H), 3038 m (N-H), 1336 s (N-O), 1618 m (C=N) (b, broad; s, strong; m, medium; w, weak).

## Synthesis of Mononuclear Cu(II) Complex ([CuL<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>])

0.3 mmole copper acetate dissolved in methanol is added 0.6 mmole of ligand in 30 mL of methanol solution. Mixture pH is adjusted to 5.5-6 with 1% KOH-alcohol while stirring at 40 °C. After 1 hr stirring stopped, precipitate filtered and washed with water and methanol. Obtained complex dried and stored.

Black compound; yield: 56%; m.p.: 112 °C. Anal. Calc. for C<sub>38</sub>H<sub>32</sub>N<sub>6</sub>O<sub>6</sub>Cu: C, 62.33; H, 4.40; N, 11.48; Cu, 8.68. Found: C, 62.45; H, 4.47; N, 11.61; Cu, 8.57%; FT-IR (KBr, cm<sup>-1</sup>): 3417 b (O-H), 3024 w (N-H), 1348 m (N-O), 1615 m (C=N), 557 w (M-O), 439 w (M-N) (b, broad; s, strong; m, medium; w, weak).

## Catecholase-like Activity

The catalytic oxidation of the 3,5-di-*t*-butylcatechol by the complex was monitored spectrophotometrically recording the increase in absorbance at 400 nm, corresponding to the formation of the *o*-quinone product 3,5-di-*t*-butylquinone (Fig. 1). Initial rate of catechol oxidation was determined by the modified method reported by Krebs [8]. The absorption at 400 nm was measured as a function of time over the first 3 min. The slope at  $t = 0$  was used to calculate the rate of the reaction.

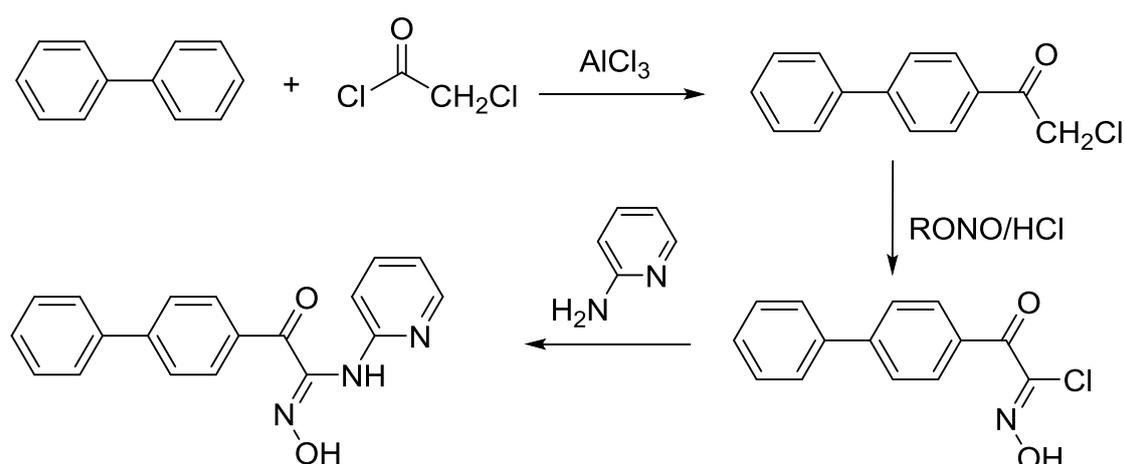
## RESULTS and DISCUSSION

The synthetic reaction steps for the ligand 2-(biphenyl-4-yl)-N'-hydroxy-2-oxo-N-(pyridin-2-yl)acetimidamide is given in Scheme 1. 1-([1,1'-Biphenyl]-4-yl)-2-chloroethan-1-one was prepared by reacting biphenyl and chloroacetyl chloride in the presence of aluminium chloride according to the Friedel–Crafts acylation [9]. 2-([1,1'-Biphenyl]-4-yl)-N-hydroxy-2-oxoacetimidoyl chloride was synthesized by the reaction of 1-([1,1'-biphenyl]-4-yl)-2-chloroethan-1-one with isopentyl nitrite in the presence of dry HCl gas [9]. 2-(Biphenyl-4-yl)-N'-hydroxy-2-oxo-N-(pyridin-2-yl)acetimidamide was originally prepared by the condensation reaction of 2-aminopyridine with ketooxime. Ligand was then reacted with appropriate metal salt, for mononuclear Cu(II) complex formation.

### Characterization of Compounds

$^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of the ligand were recorded in  $\text{CDCl}_3$  solution. Deuterium exchangeable protons of the ligand showed a chemical shift at 9.81 and 8.69 ppm as a singlet peak for  $-\text{C}=\text{N}-\text{OH}$  and  $-\text{NH}-$  group, respectively. In addition, aromatic protons of the ligand were observed between 7.45-8.08 ppm as a multiplet peak.

Chemical shift for carbonyl carbon was observed at 182.13 ppm in  $^{13}\text{C}$ -NMR spectrum of the ligand. Carbon atoms in  $-\text{C}=\text{N}-$  of oxime and pyridine were obtained at 154.27 and 163.48 ppm, respectively. The carbon resonances of aromatic groups were exhibited shifts between 126.74 and 163.48 ppm. The NMR results are all in good agreement with the proposed structure for the ligand [10-12].



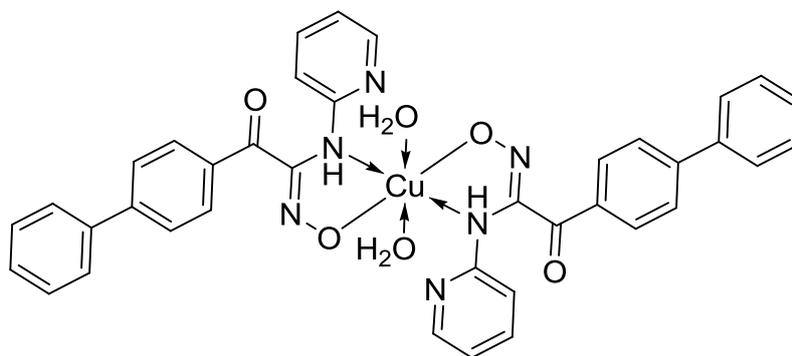
**Scheme 1.** Synthetic route for the synthesis of the ligand (HL)

In the FT-IR spectrum of the ligand, O-H stretching vibration of oxime group in ligand observed at  $3288\text{ cm}^{-1}$  and this broad band was disappeared by complex formation which indicates participation of oxime oxygen in the coordination. Broad peaks appearing between  $3417\text{ cm}^{-1}$  in the spectrum indicate that  $\text{H}_2\text{O}$  in the metal complex is coordinated to the Cu(II) ion. The band attributed to N-H stretching vibration observed at  $3038\text{ cm}^{-1}$  was shifted to  $3024\text{ cm}^{-1}$  due to the complex formation. This shift indicated the participation of nitrogen atom of N-H group in the complex. Stretching band at  $1336\text{ cm}^{-1}$  in the FT-IR spectrum of the free ligand can be attributed to the N-O bond of oxime group. This band was shifted to the  $12\text{ cm}^{-1}$  higher frequency which indicates the formation of coordination bond between metal and oxygen atom of oxime group [13,14]. The coordination mode of the ligand is further supported by new bands appeared at  $557$  and  $439\text{ cm}^{-1}$  which have been assigned tentatively to  $\nu(\text{M-O})$  and  $\nu(\text{M-N})$ , respectively [15].

The molar conductivity of metal complex in N,N-dimethylformamide ( $10^{-3}\text{ M}$ ) measured at room temperature was  $21\ \Omega^{-1}\text{cm}^2\text{mol}^{-1}$  which points out the non-electrolyte nature of chelate [16].

Magnetic susceptibility measurement of the complex was carried out in solid state at ambient temperature. It was found that Cu(II) complex is paramagnetic and observed magnetic moment value is 1.87 BM. The magnetic data of the Cu(II) complex was in accordance with a  $d^9$  metal ion.

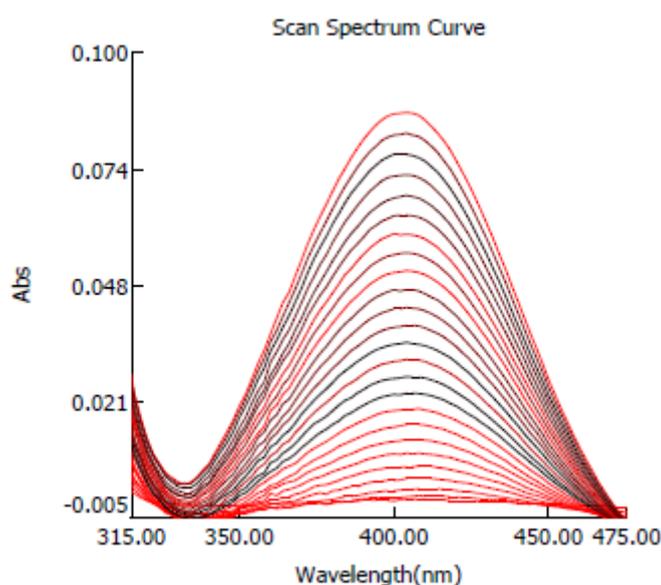
The elemental and ICP-OES analyses of the complex indicate that the metal:ligand ratio is 1:2. According to FT-IR, elemental analyses, ICP-OES results, molar conductivity and magnetic susceptibility measurements, the Cu(II) complex have an octahedral structure.



**Figure 2.** Proposed structure for the synthesized complex

## Catecholase-like Activity Studies

Catecholase-like enzyme activity of the Cu(II) complex was investigated using 3,5-di-*t*-butylcatechol as a substrate. Complex oxidizes 3,5-di-*t*-butylcatechol to the 3,5-di-*t*-butylquinone in aerobic conditions which can be followed spectrophotometrically by the increase in absorbance at 400 nm which is specific for the product. The oxidation reaction cannot be observed in the absence of oxygen in reaction medium. Enzyme activity measurements were carried out in methanol due to good solvation of the substrate and its product. It was found that synthesized complex possess a catalytic efficiency in oxidation reaction of 3,5-di-*t*-butylcatechol (Fig. 3).

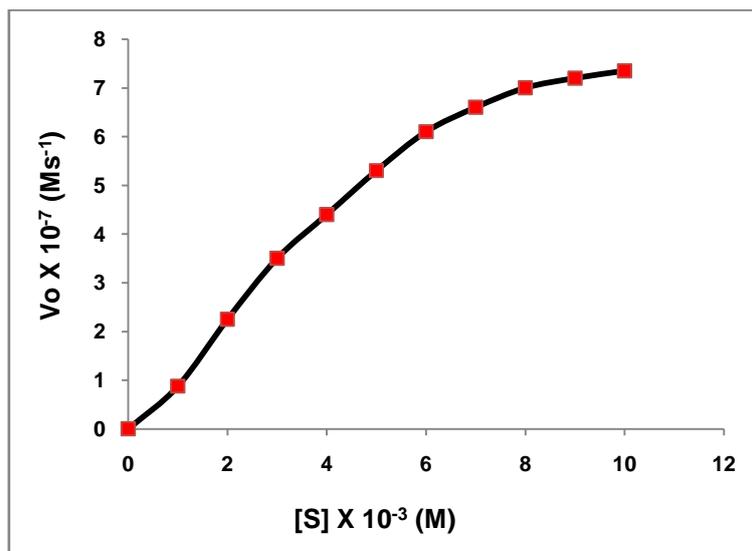


**Figure 3.** Spectral scan of 3,5-di-*t*-butylquinone taken by 30 sec. intervals

$$[\text{substrate}] = 5 \times 10^{-3} \text{ M}, [\text{complex}] = 1 \times 10^{-4} \text{ M}$$

Kinetic parameters of oxidation reaction were obtained from additional experiments. A linear relationship between the initial rates and complex concentration was observed when the substrate concentration was fixed and complex concentration varied. Linear relationship indicates a first-order dependence of the rate on the catalyst concentration. Additionally, the Cu(II) complex was treated with varying concentrations of 3,5-di-*t*-butylcatechol. A first-order dependence was obtained from the lower substrate concentrations of 3,5-di-*t*-butylcatechol. However, at higher concentrations, the complex was showed saturation

kinetics due to its lower initial rate values. Michaelis–Menten approach was applied for the determination of kinetic data.



V<sub>max</sub> and K<sub>m</sub> values for Cu(II) complex were obtained as (9.21x10<sup>-7</sup> Ms<sup>-1</sup>) and (12.7 M), respectively. Furthermore, turnover number (k<sub>cat</sub>) was also calculated as 66 (h<sup>-1</sup>) which is comparable with other catecholase model complexes [17,18].

## CONCLUSION

Originally synthesized bidentate ligand coordinated oxygen atom of oxime group and nitrogen atom of –NH– group bonded to aromatic ring. Two ligands were ligated to one Cu(II) ion and formed octahedral geometry with the participation of two coordination water molecules. Catecholase-like enzyme activity was commonly studied by the dinuclear metal complexes. In this study, finally constructed mononuclear metal complex showed a high catecholase-like enzyme activity comparable to dinuclear complexes.

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