

Article

Synthesis, Structure and Catechol Oxidase Activity of Mono Nuclear Cu(II) Complex with Phenol-Based Chelating Agent with N, N, O Donor Sites

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Abstract: A square-planar Cu(II) complex [Cu(L)Cl], **1**, with sterically constrained tridentate phenol-based ligand (HL= *N,N,N'*-trimethyl-*N'*-(2-hydroxy-3,5-di-*tert*-butylbenzyl)-ethylenediamine) with *N, N, O* donor sites has been synthesized. The complex is characterized by single crystal X-ray diffraction study as well as other spectroscopic techniques. The reported complex crystallizes in monoclinic space group *C2/c* with *a* = 30.248(6), *b* = 13.750(3) and *c* = 11.410(2) Å with $\beta = 110.232(2)^\circ$. The Cu(II) ion adopts a square planar environment in this complex. Electrochemical study of the complex **1** gives quasi-reversible reductive response at $E_{1/2} \approx -0.5$ V due to the reduction of the Cu(II) center along with a reversible oxidation peak at $E_{1/2} \approx 0.75$ V. The oxidation peak arises due to the ligand-based oxidation of phenolate group to phenoxyl radical in the complex. The Cu(II) complex exhibits catechol oxidase activity in methanol as observed by the UV-vis spectroscopy of the aerial oxidation of 3,5-DTBC to 3,5-DTBQ and the reaction proceeds via the formation of ligand phenoxyl radical. The turnover number for complex **1** is 2560 h⁻¹.

Keywords: Cu(II) complex; tridentate ligand; syntheses; characterization; X-ray structure; catecholase activity



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1. Introduction

It is now well established that metalloenzymes with Cu(II) ion at an active site can act as redox catalyst in many biological processes which include electron transfer, reduction of nitrogen oxides, metal ion uptake, storage and transport, di-oxygen activation and transport as well as oxidation of different organic substrates [1]. One of the most scientifically published copper containing metalloenzymes is catechol oxidase which is a type-3 copper containing metalloenzyme. This type of enzyme is usually found in plant tissues as well as in fungi and bacteria and catalyzes oxidation of catechol under aerobic conditions that results in the generation of quinone. These quinones undergo auto-polymerization reaction to generate brown poly-phenolic pigment melanin which is responsible for protecting plant's tissues from damage against pathogens and insects [2]. The crystal structure of this di-copper enzyme has been published by B. Krebs et al. in 1998. This metalloenzyme contains a hydroxo bridged di-copper(II) center and each Cu(II) center is coordinated to three histidine ligands [3]. Numerous di-copper model compounds exhibiting catechol oxidase activity have been reported [4]. Some reported mono-copper complexes also show catecholase activity [5].

Metal complexes with redox active ligand have been considered of great interest in various fields of inorganic chemistry such as coordination chemistry, catalysis and bio-inorganic chemistry [6]. It has been observed that electrochemically active ligands play

a crucial role in natural systems [7]. It is also documented that phenoxyl radical generated during galactose oxidase catalytic cycle [8]. Various research groups have reported that metal complexes with redox active ligands are used in organic transformation reaction [9].

We report a mononuclear square-planar Cu(II) complex [Cu(L)Cl], **1**, with tridentate redox active ligand HL with *N, N, O* donor sets and a chloride as an ancillary ligand. This mononuclear square-planar complex **1** (CCDC 2075674 contains the supplementary crystallographic data for complex **1**.) has been characterized by various spectroscopic tools. Molecular structure of this Cu(II) complex was determined by single crystal X-ray diffractometer. Cyclic voltammogram of complex **1** in methanol shows a quasi-reversible peak at $E_{1/2} \approx -0.5$ V, originated due to the reduction of Cu(II) center and one reversible oxidation peak at $E_{1/2} \approx 0.75$ V due to the ligand centered redox process in which the phenolate group transforms phenoxyl radical in this complex.

The square-planar copper complex exhibits catecholase activity at room temperature in methanol. Kinetic studies suggest that the turnover number of complex **1** is 2560 h⁻¹. By monitoring time dependent UV–visible spectroscopy, cyclic voltammetry of complex **1** and identification of hydrogen peroxide in the catalytic process, the oxidation of 3,5-DTBC to 3,5-DTBQ in the presence of air occurs via the formation of phenoxyl radical in the catalytic cycle.

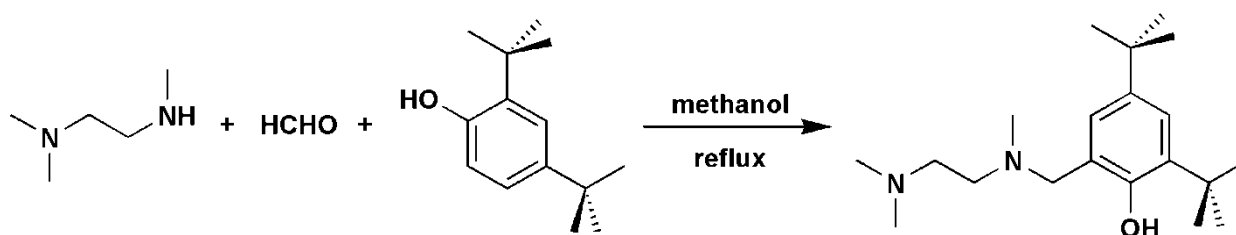
2. Materials and Methods

2.1. Materials

N,N,N'-tri-methyl ethylenediamine, 2,4-di-*tert*-butylphenol and 3,5-DTBC were received from Aldrich (Sigma Aldrich, St Louis, MO, USA). Solvents were reagent grade and were purified using appropriate drying agents before use [10]. Other chemicals were also of reagent grade, and we used these without further purification.

2.2. Syntheses of *N,N,N'*-trimethyl-*N'*-(2-hydroxy-3,5-di-*tert*-butylbenzyl)-ethylenediamine(HL)

A total of 10 mmol (1.02 g) of *N,N,N'*-trimethyl ethylenediamine was taken in 50 mL methanol (Scheme 1). To the methanolic solution, about 0.30 g (10 mmol) of paraformaldehyde was mixed. The reaction mixture was then refluxed for 3 h. A total of 10 mmol (2.06 g) of 2,4-di-*tert*-butylphenol was added to it. The resulting mixture was again refluxed for another 12 h to obtain an orange solution. The solvent evaporated to obtain gummy material which, on keeping several days in a vacuum, produced light yellow hygroscopic material. Recrystallization of the compound was unsuccessful after several attempts.



Scheme 1. Schematic presentation for synthesis of the ligand HL.

The ligand was then reacted with metal salt without further purification.

2.3. Preparation of [Cu^{II}(L)Cl] **1**

A total of 0.16 g (0.5 mmol) of ligand HL was taken in 25 mL methanol. Few drops of triethylamine were added to it. A total of 0.085 g (0.5 mmol) of CuCl₂·2H₂O was mixed to this reaction mixture and then stirred for 1 h. Resulting solution was finally filtered and filtrate was left undisturbed in open air for crystallization. A brown color crystalline compound along with diffraction quality single crystal was obtained. It was collected by filtration. Yield: 0.10 g (50%). IR (KBr disk cm⁻¹): 3446, 3360, 2948, 2738, 2676, 2491, 1473, 1314, 1288, 1169, 1032, 830, 620, 542, 513, 490. Anal. Calcd. for C₂₀H₃₅ClCuN₂O: C, 57.4; H,

8.4; N, 6.7. Found: C, 57.0; H, 8.1; N, 6.0. UV-Vis (MeOH) [λ_{\max}/nm] ($\epsilon_{\max}/\text{mol}^{-1} \text{cm}^2$): 486 (549), 685 (175). ESI-MS in CH_2Cl_2 : m/z ($\text{M}^+ \text{Li}^+$); 421.

2.4. Physical Measurements

A Perkin-Elmer 950 UV/VIS/NIR spectrophotometer (San Diego, CA, USA) was used to record UV-visible spectra in solution. To record IR spectra, a Nicolet Magna 750 FT-IR spectrometer, series II (San Diego, CA, USA), was utilized, where KBr was applied as medium. Mass spectra (ESI-MS in positive ion mode) was collected on a QTOF Model YA263 Micro Mass Spectrometer (Waters, Manchester, UK). Electrochemical experiments were completed with a BASi epsilon electrochemistry instrument (Austin, TX, USA). For this measurement, a three-electrode assembly comprising of a Pt electrode (for oxidation process), glassy carbon (for reduction reaction), a Pt auxiliary electrode and Ag/AgCl reference electrode were utilized. Cyclic voltammetric measurements were carried out at 25 °C in acetonitrile solution of Cu(II) complex **1** (Ca.1 mM). In this study, 0.1 M solution of tetraethyl ammonium perchlorate (TEAP) was added as supporting electrolyte in order to maintain sufficient conductivity.

2.5. X-ray Crystallography

Diffraction quality crystals of Cu(II) complex **1** were obtained by slow evaporation of reaction mixture at room temperature. Intensity data were collected on a Bruker APEX II CCD (Bruker, Karlsruhe, Germany) diffractometer at 150 K. Intensity data were measured with $\theta_{\max} = 25.65^\circ$. No crystal decay was found during data collection. Relevant crystal data and refinement details of complex **1** are shown in Table 1. Structure was determined by using direct methods [11] and refined on F^2 , applying full-matrix least-square procedure by SHELXL 97 program (Release 1, Program for the Refinement of Crystal Structure. *Shelxl-97 Program Cryst. Struct. Refinement* 1997) [12].

Table 1. Crystallographic data for complex **1**.

Empirical Formula	$\text{C}_{20}\text{H}_{35}\text{ClCuN}_2\text{O}$
Formula weight	418.49
T (K)	150(2)
λ (Å)	0.69230 Å
Space group	monoclinic
Crystal system	$\text{C}_{2/c}$
a (Å)	30.248(6) Å
b (Å)	13.750(3) Å
c (Å)	11.410(2) Å
α	90°
β	$110.232(2)^\circ$
γ	90°
V (Å ³)	4452.7(15)
Z	8
μ (mm ⁻¹)	1.111
F (000)	1784
θ ranges (°)	2.55–25.65°
Index ranges	$-37 \leq h \leq 37$
	$-17 \leq k \leq 17$
	$-14 \leq l \leq 14$

Table 1. Cont.

Empirical Formula	C ₂₀ H ₃₅ ClCuN ₂ O
Reflections collected	18177
Goodness of fit	1.039
No. of parameters	245
R1(F _o) ^a , wR2(F _o) ^b (all data)	0.0683, 0.1359
Largest diff. peak, deepest hole (eÅ ⁻³)	0.433 and -0.391

$$^a R = \frac{\sum ||F_0| - |F_c||}{\sum |F_0|}$$

$$^b wR = \left[\frac{\sum [w(F_0^2 - F_c^2)]}{\sum w(F_0^2)} \right]^{1/2}$$

2.6. Catecholase Activity

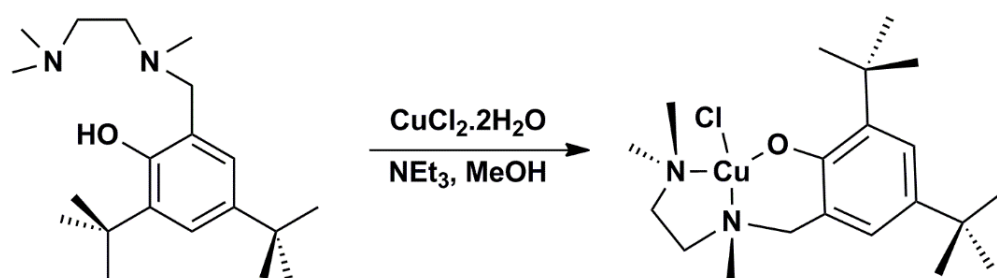
Catecholase activity of Cu(II) complex **1** was examined by taking 3,5-di-*tert*-butyl catechol (3,5-DTBC) as a model substrate [13]. Oxidation reaction was performed in methanol at room temperature under aerobic condition. In order to check catecholase activity of the Cu(II) complex, 100 equivalent of substrate viz., 3,5-DTBC was mixed with 10⁻⁴ M methanolic solution of mononuclear Cu(II) complex **1**. Course of the reaction was followed spectrophotometrically by watching increase in absorbance of quinone band near 400 nm [14].

To find out dependence of reaction rate on concentration of substrate as well as upon different experimental parameters, almost 1.75 × 10⁻⁴ M solution of Cu(II) complex **1** was mixed with increasing amount of 3,5-DTBC. In each case, the reaction was followed spectrophotometrically by monitoring increase in absorbance at around 400 nm up to 6 h. Michaelis–Menten method of enzymatic kinetics was applied to obtain Lineweaver–Burk plot and to obtain values of K_M, V_{max} and K_{cat}. Hydrogen peroxide formed during this catalytic reaction was identified by modified iodometric method [15].

3. Results and Discussions

3.1. Synthesis

Mononuclear Cu(II) complex **1** has been synthesized by stirring CuCl₂·2H₂O with the methanolic solution of phenol-based ligand HL containing N, N, O donor sites. Schematic diagram for complex preparation is depicted in Scheme 2. Cu(II) occupies square-planar environment in this complex as revealed from single crystal X-ray structure. Characteristic bands of the coordinated ligand anion have been found in IR spectrum of complex **1**. A prominent band appears at 1288 cm⁻¹ due to ν(c-o/phenolate) stretching vibration [16]. Cu-O and Cu-N bands arise at 513 and 490 cm⁻¹, respectively, as compared with reported papers [17]. The ESI mass spectrum (positive ion mode) of complex **1** shows the molecular ion peak at m/z = 421 (M⁺Li⁺) in CH₂Cl₂ medium (Figure S1).

Scheme 2. Schematic presentation for synthesis of complex **1**.

3.2. Description of Crystal Structure

Molecular structure of Cu(II) complex **1** is given in Figure 1. Selected bond distance and angles are tabulated in Table 2. Cu(II) complex **1** crystallizes in monoclinic space group $C_{2/c}$. Cu(II) center in this mononuclear complex is surrounded by N(1), N(2) and O(1) donor sites of deprotonated tridentate ligand (L)[−] and one ancillary Cl[−] ligand in nearly square-planar arrangement. Bond distances of Cu–O (1.824(4) Å) and Cu–N (2.024(3) – 2.088(3) Å) are all in the expected region [18].

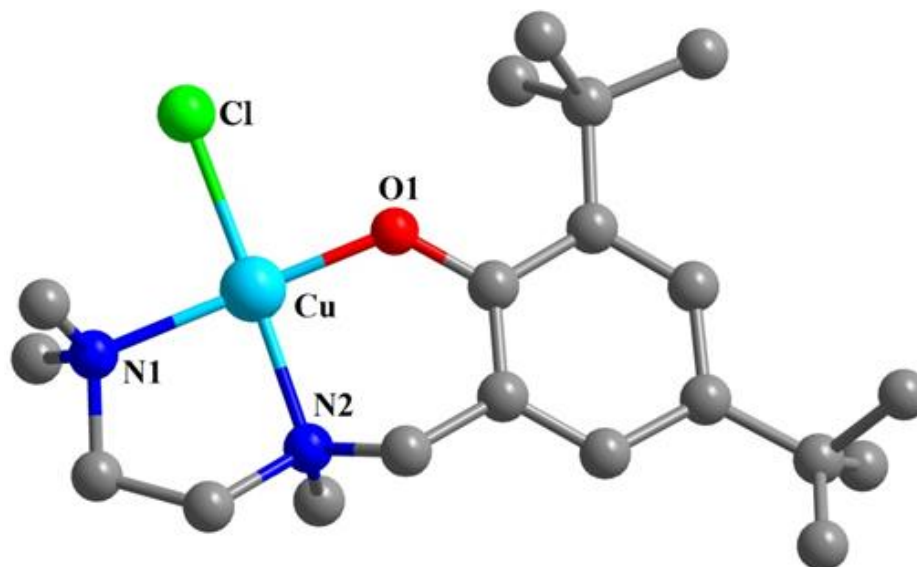


Figure 1. ORTEP diagram of complex **1**. Disordered and H-atoms are omitted for clarity.

Table 2. Selected bond distance (Å) and angles (deg) for complex **1**.

Bond Distances (Å)		Bond Angles (deg)	
Cu(1)–Cl(1)	2.2475(11)	Cl(1)–Cu(1)–N(1)	92.52(10)
Cu(1)–N(2)	2.024(3)	Cl(1)–Cu(1)–N(2)	177.52(10)
Cu(1)–N(1)	2.088(3)	Cl(1)–Cu(1)–O(1)	88.45(15)
Cu(1)–O(1)	1.824(4)	N(1)–Cu(1)–N(2)	85.57(13)
		N(1)–Cu(1)–O(1)	170.14(18)
		N(2)–Cu(1)–O(1)	93.17(17)

Magnitude of τ_4 value, to quantify extent of distortion from ideal square-planar structure, for this copper compound, is obtained as 0.0875 [19]. Here, the Cl(1)–Cu(1)–N(2) angle (177.52) and N(1)–Cu(1)–O(1) angle (170.14) have been taken as α and β , respectively.

3.3. Electronic Spectra

UV–visible spectra of the 1×10^{-4} M copper complex **1** has been recorded in methanol at room temperature, Figure 2. Complex **1** shows two absorption bands at 486 and 685 nm. These bands may be assigned to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ and ${}^2B_{1g} \rightarrow {}^2E_{1g}$ transitions, respectively, as expected for Cu(II) complex [20]. Remaining band maxima appearing around UV region may be assigned to intra-ligand charge transfer transitions.

3.4. Electrochemical Studies

Electrochemical properties of square-planar Cu(II) complex **1** have been monitored by cyclic voltammetry in methanol solution (0.1 M TEAP). Cyclic voltammogram of complex **1** is displayed in Figure 3. It shows one reductive response at $E_{1/2} \approx -0.5$ V, which is quasi-reversible in nature and arises due to reduction of Cu(II) center [21]. One reversible oxidation peak for [Cu(L)Cl], **1**, also appears at $E_{1/2} \approx 0.75$ V, which is due to oxidation of phenolate group to phenoxyl radical in the complex as reported earlier [22].

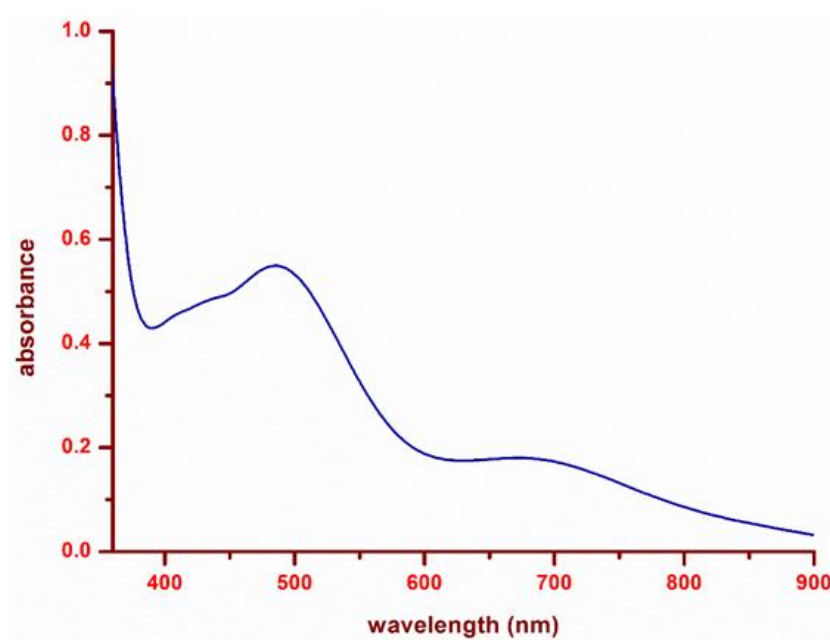


Figure 2. Electronic absorption spectra of complex 1 in MeOH.

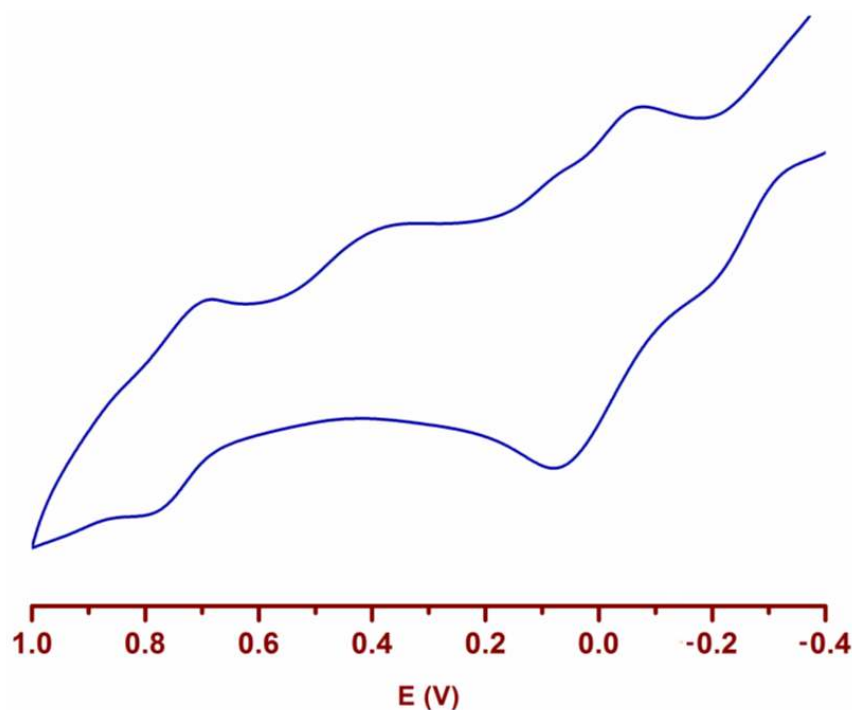


Figure 3. Cyclic voltammogram of complex 1 in methanol.

3.5. Catecholase Activity

Square-planar Cu(II) complex 1 shows catechol oxidase activity in methanol at room temperature. Its catecholase activity was examined using 3,5-di-tert-butylcatechol (3,5-DTBC) as a model substrate. 3,5-di-tert-butylcatechol was oxidized to 3,5-di-tert-butylquinone (3,5-DTBQ) in the presence of Cu(II) complex 1 under aerobic condition in methanol. Formation of quinone was confirmed by monitoring a characteristic absorption band around 400 nm in methanol, as shown in Figure 4. In this process, molecular oxygen was reduced to produce hydrogen peroxide that was investigated by modified iodometric method. Rate constant for a certain complex–substrate concentration ratio was examined by change in absorbance versus time plot by using initial rate method. Dependence of reaction

rate on concentration of 3,5-DTBC, i.e., substrate, was monitored by reacting 1.75×10^{-4} M solution of Cu(II) complex 1 with an increasing amount of 3,5-DTBC from 1.5×10^{-3} to 5.3×10^{-3} M in methanol in the presence of air. Observed rate vs. [substrate] plot in methanol solution and Lineweaver–Burk plot are depicted in Figure 5. Observed rate vs. substrate concentration data are then examined based on Michaelis–Menten approach of enzymatic kinetics in order to calculate values of V_{\max} , K_M and K_{cat} . Details of different enzyme kinetic parameters are written on Table 3.

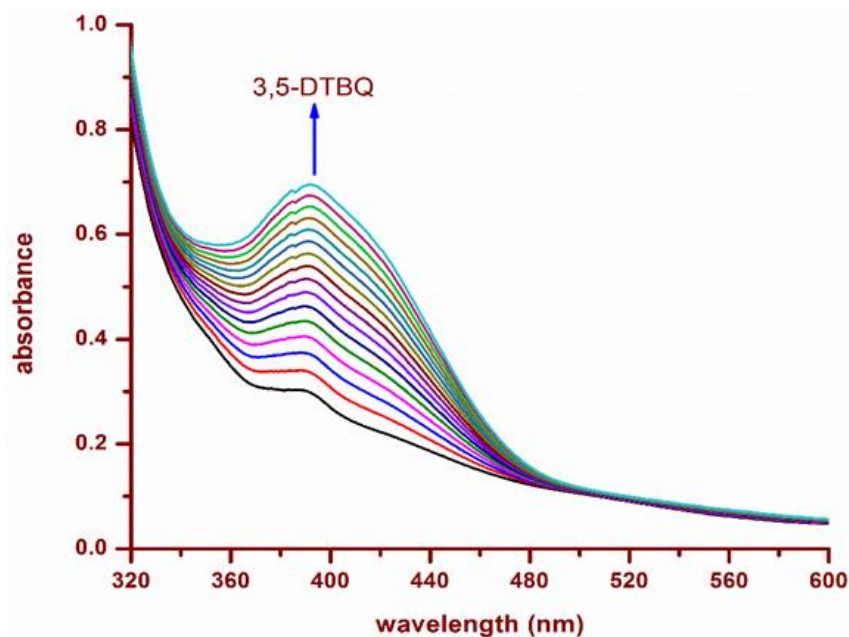


Figure 4. Increase in absorbance of quinone band at around 400 nm after addition of 3,5-DTBC to a methanolic solution of complex 1.

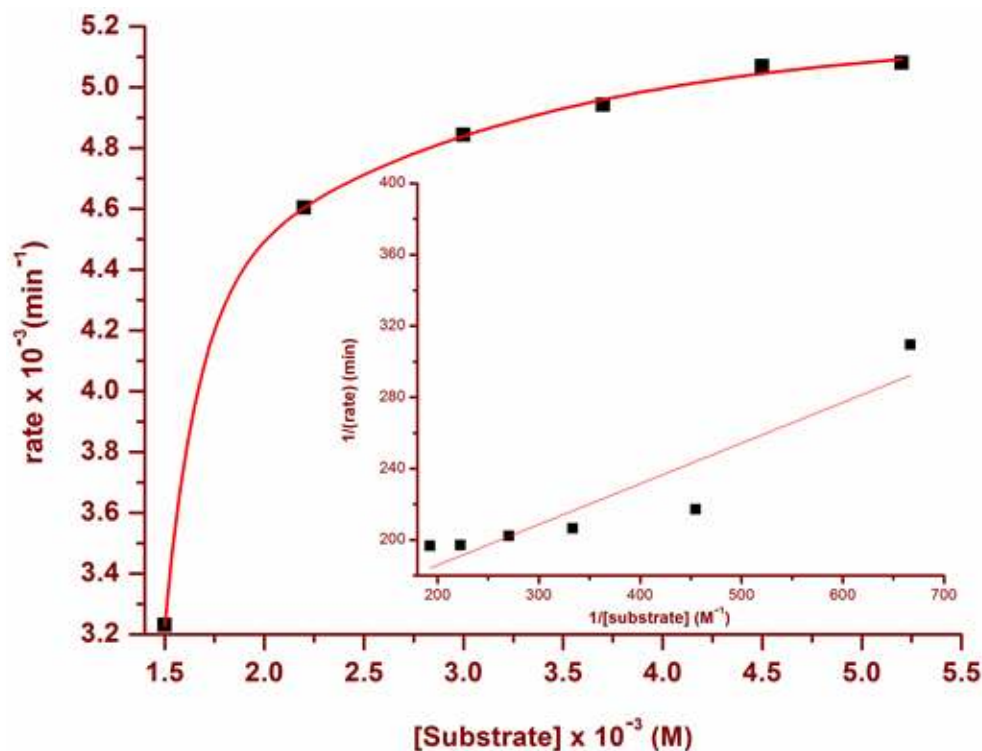


Figure 5. Plot of rate vs. [substrate] in the presence of 1 in MeOH; inset represents Lineweaver–Burk plot.

Table 3. Kinetic parameters for oxidation of 3,5-DTBC catalyzed by **1**.

Solvent	Complex	V_{\max} (M min ⁻¹)	Std. Error	K_M (M)	Std. Error	k_{cat} (h ⁻¹)
MeOH	1	7.13×10^{-3}	5.64×10^{-2}	1.63×10^{-3}	2.38×10^{-3}	2560

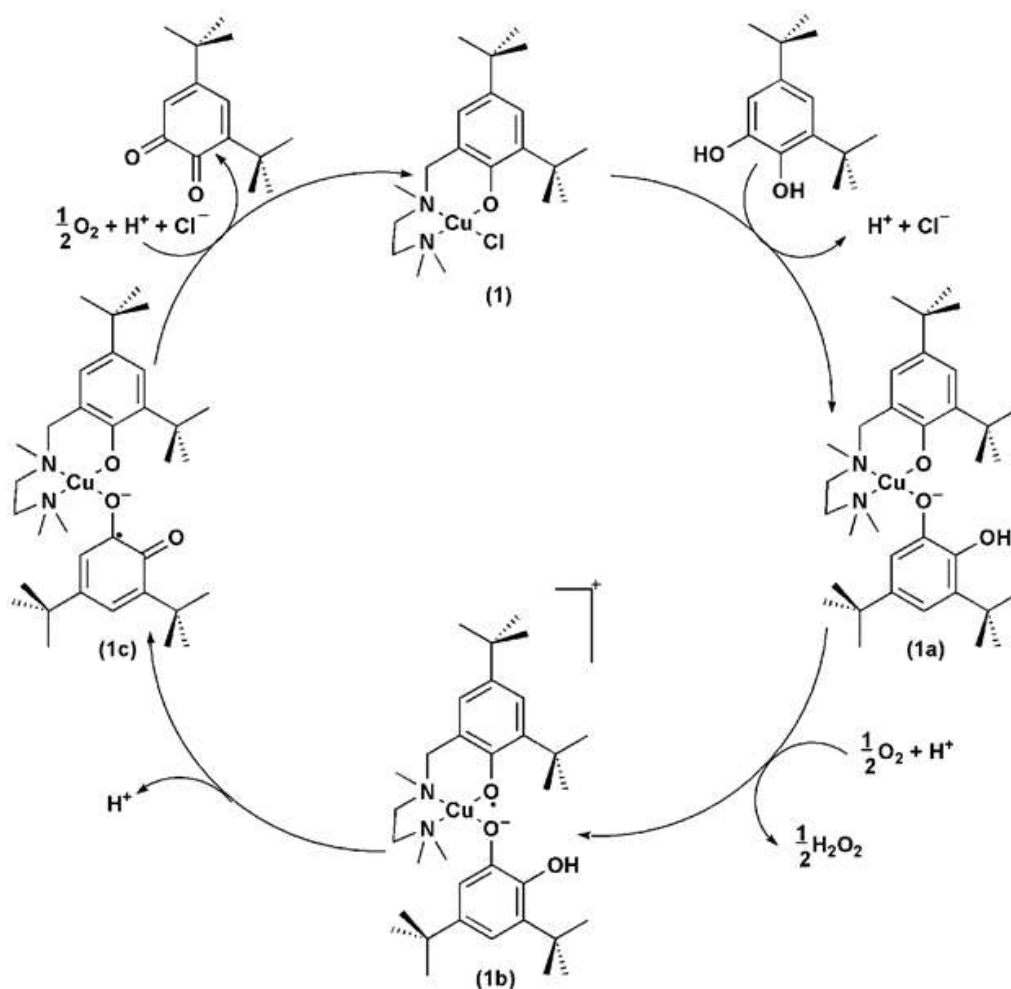
From this comparison of catecholase activity among different mono and dinuclear complexes of copper, one can infer that complex [CuII(L)Cl], **1**, is an effective catalyst for oxidation of 3,5-DTBC to 3,5-DTBQ (Table 4) [23–26].

Table 4. Comparison of K_{cat} value for oxidation of 3,5-DTBC to 3,5-DTBQ by various copper complexes.

Complex	Solvent	V_{\max} (M min ⁻¹)	K_M (M)	K_{cat} (h ⁻¹)	Ref.
[Cu(H ₂ L ^a)(HL)]ClO ₄	methanol	1.52×10^{-2}	2.62×10^{-3}	9.11×10^3	23
[Cu(H ₂ L ^a)(HL)Cu(L)] ⁺	methanol	3.46×10^{-4}	1.20×10^{-3}	2.08×10^4	23
[Cu ₂ (H ₂ -bbppnol)(μ-OAc)(H ₂ O) ₂]Cl ₂ ·2H ₂ O	methanol	1.44×10^{-5}	7.9×10^{-4}	2.84×10^1	24
[Cu ₂ (Hbtppnol)(μ-OAc)](ClO ₄) ₂	methanol	1.44×10^{-5}	9.5×10^{-4}	2.81×10^1	24
[Cu ₂ (P1-O)(OAc)](ClO ₄) ₂	methanol	4.02×10^{-5}	8.6×10^{-4}	1.08×10^1	24
[Cu ₂ L ₂ ^b (ClO ₄) ₂]	methanol	1.56×10^{-4}	3.32×10^{-3}	9.36×10^2	25
[Cu ₂ L ₂ ^b (OH)]ClO ₄	methanol	3.89×10^{-4}	4.60×10^{-3}	2.33×10^2	25
[Cu ₂ L ^c]	methanol	6.74×10^{-3}	1.17×10^{-2}	7.22×10^2	26
[Cu ^I (L)(Cl)], 1	methanol	7.13×10^{-3}	1.63×10^{-3}	2.56×10^3	Present work

Where H₂L^a = Pyridine-2,6-dimethanol, H₃-bbppnol = *N,N'*-bis(2-hydroxybenzyl)-*N,N'*-bis-(pyridylmethyl)-2-hydroxy-1,3-propanediamine, H₂btpnol = *N*-(2-hydroxybenzyl)-*N,N',N'*-tris(2-pyridylmethyl)-1,3-diaminopropan-2-ol, P1-OH = 1,3-bis[bis(2-pyridylmethyl) amino] propanol, HL^d = 2-[[2-(diethylamino)-ethylamino]methyl]phenol, H₂L^c = *N,N'*-bis(2-hydroxy-3,5-di tertiarybutylbenzyl)- homopiperazine.

We have proposed a plausible mechanism for generation of 3,5-DTBQ from 3,5-DTBC catalyzed by complex **1** in the presence of air. Catalytic cycle is described in Scheme 3. In the first step of the catalytic cycle, Cu(II) complex, **1**, releases chloride from its coordination zone and simultaneously binds with substrate molecule to form species **1a**. Molecular oxygen oxidizes **1a** to produce redox active phenoxyl radical species **1b**, in the next step of this proposed catalytic cycle. We observe a band around 702 nm and their gradual increase with time (Figure S2), and such phenomenon indicates that phenoxo radical species are most probably generated during the catalytic cycle as compared with reported paper [27]. Cyclic voltammetry study of complex **1** provides additional support of this incident. Here, molecular oxygen itself is reduced to hydrogen peroxide and then the reaction moves through generation of Cu(II)-semiquinonate radical species **1c**. In the last step, Cu(II)-semiquinonate radical species **1c** reacts with molecular oxygen in the presence of chloride ligand to generate 3,5-DTBQ along with H₂O₂ as a by-product, and the catalytic cycle is completed with formation of mononuclear Cu(II) complex, **1**. It is proved that nearly one mole of hydrogen peroxide is generated per mole of 3,5-DTBC consumed in the catalytic cycle [28].



Scheme 3. A plausible mechanistic route for catechol oxidation catalyzed by complex **1** in presence of air.

4. Conclusions

A square-planar Cu(II) complex, **1**, with a redox active tridentate phenol-based ligand with *N, N, O* donor sites has been reported. Structure of complex **1** has been determined by single crystal X-ray diffraction study. The mononuclear Cu(II) complex **1** crystallizes in monoclinic space group $C_{2/c}$. Electrochemical study reveals that Cu(II) complex **1** undergoes one electron quasi-reversible reduction at $E_{1/2} \approx -0.5$ V vs. Ag/AgCl electrode due to Cu(II)/Cu(I) electron transfer and one reversible peak arises at $E_{1/2} \approx 0.75$ V due to ligand-based oxidation of phenol moiety to phenoxyl radicals. Cu(II) complex **1** effectively catalyzes aerial oxidation of 3,5-di-*tert*-butylcatechol (3,5-DTBC) in methanol. The reaction follows the Michaelis–Menten enzymatic reaction kinetics in methanol. K_{cat} value for this reaction is 2.56×10^3 h⁻¹. The oxidation reaction occurs via radical generation in the catalytic cycle. UV-visible spectrophotometric study and electrochemical experiment supports that the conversion of 3,5-DTBC to 3,5-DTBQ occurs via ligand-based oxidation of phenolate moiety in the complex **1**.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/cryst12040511/s1>, Figure S1: Mass spectra of complex **1** in dichloromethane, Figure S2: Increase in the phenoxo radical species absorption at around 702 nm upon addition of 3,5-DTBC to an methanolic solution of complex **1**.

Author Contributions: Conceptualization, R.G. and D.M. (Debdas Mandal); methodology, T.R. and B.M.; formal analysis, T.R.; investigation, A.H.; data curation, D.M. (Dhrubajyoti Mondal);

writing—original draft preparation, D.M. (Dhrubajyoti Mondal); writing—review and editing, R.G.; supervision, D.M. (Debdas Mandal); funding acquisition, D.M. (Debdas Mandal) All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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