Tuning the structural and catalytic properties of copper(II)-based complexes containing pyridine-2,6-diimines

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Communicated by Ramaswamy H. Sarma

ABSTRACT

Four mononuclear penta coordinated copper(II) chelates, [CuLBr2] nH2O, containing the tridentate neutral ligands, pyridine-2,6-diimine (L), were prepared via the template technique. Analytical and several physicochemical methods have been used to characterize the prepared metal chelates. Square-pyramidal stereochemistry was described to the current copper(II) complexes. DFT technique has been applied to optimize the structure of the running diimines and their corresponding copper-based compounds. Ligand substitution study performed to link the catalytic potency of the candidate oxidase mimics and their lability characters. Spectral investigations reveal that nature of substituents of the chelated ligands effectively tuning the Lewis acidity of copper(II) centers. Biomimetics of redox proteins specifically containing copper were examined towards the aerobic oxidation of polyphenol. Kinetic studies with the stopped-follow technique showed a close association between the Lewis acidity of the copper(II) nuclei of the prepared chelates and their oxidase-like activity. The catalytic activity of the natural enzyme (catechol oxidase from sweet potatoes) measured and compared with that for the present CuII chelates. The thermodynamic parameter drive force (ΔG° or λ) of the performed oxidation processes was determined from the values of redox potential of the chemical species involved in these catalytic reactions. The proposed catalytic reactions pathways have been discussed based on the outcomes of the kinetic investigations.

1. Introduction

The presence of copper ion in some metalloproteins as an active center gives them the property of redox catalysts and the ability to participate in many biological processes such as electron transport and oxidation of some biologically important substrates (Bertini et al., 2007). Of the metalloenzymes that catalyze redox reactions of poly-phenols are tyrosinase and catechol oxidase enzyme, which contains copper as an active site (Bertini et al., 2007).

The three nitrogen atoms of histidine residues are the coordination sites attached to the copper centers of these enzymes (Solomon et al., 1996; 1992). A distinctive feature of these copper proteins is the ability to bind inversely with atmospheric oxygen (Bertini et al., 2007). In this regard, copper center of catechol oxidase protein catalyzes the aerobic transformation of wide verities of ortho diphenols to biologically significant quinones (Rompel et al., 1999). Afterwards, the highly reactive ortho quinones undergo an automatic polymerization process that leads to the formation of melanin pigment, which is thought to be a line of defense against pathogens or insects that may attack the torn tissue (Deverall, 1961). The main difference between the aforementioned plant redox enzymes, catechol oxidase and tyrosinase is that the latter catalyzes ortho hydroxylation of the amino acid tyrosine in a process known as cresolase. This process is followed by the oxidation of the formed ortho diphenol into an ortho quinone (catecholase process).

As is known, catechol oxidase catalyzes the aerobic transformation of ortho diphenols to ortho-quinones with the formation of two water molecules through a four-electron reduction of O2 (the catecholase process). In the same context, the oligomeric multicopper protein, phenoxazinone synthase, is another redox copper enzyme that is functionally similar to catechol oxidase. It aerobicly catalyzes the transformation of ortho-amino phenol to the biologically significant product ortho-amino-3H-phenoxazine-3-ones (APX). APX is produced naturally at the end of the bacterial synthesis process for the antibiotic Actinomycin D.

Over the past decades, metal chelates containing 2,6-bis-(imino)pyridyl based ligands with three-donor nitrogen atoms and their similar ligands have acquired interest due to their use in catalysis and many applications (Archer et al., 2006; Bianchini et al., 2000; Bouwkamp et al., 2006; Britovsek...
et al., 1999; Chen et al., 2003; Gibson et al., 2007; Ittel et al., 2000; Paulino & Schuchardt, 2004; Small et al., 1998; Van der Boom & Milstein, 2003). This family of diimines is characteristic by rigid pyridine backbone and two imine side arms, which provide three coplanar nitrogen donors. The importance of pyridine-containing metal complexes is due to the similarities with nitrogenous bases commonly found in the structures of redox enzymes and their tuning role of the catalytic processes. In light of this, several works have addressed these versatile ligands and their effect on the catalytic performance of their metal complexes (Bianchini et al., 1999; Britovsek et al., 2005, 2003; Çetinkaya et al., 1999; Gao et al., 2009; Gibson et al., 2007; Mohamed, 2006; Small et al., 1998).

With regard to metal complexes, which contain Schiff bases, the introduction of electron donating or electron withdrawing substituents into the pyridine ring will have a great influence on their electronic properties and this will reflect on their catalytic performance. This is because the catalytic action of these systems is strongly dependent on the intense interaction between the electrons of the π-orbital and the completely unfilled d-orbital of the transition metal center (Bart et al., 2006; Britovsek et al., 2005, 2003; Çetinkaya et al., 1999; Gao et al., 2009; Gibson et al., 2007; Mohamed, 2006; Small et al., 1998).

In situ, new tridentate pyridine-2,6-diimine (L1–4) as copper(II) chelates were prepared via Schiff condensation in presence of copper(II) ion. The analytical and thermal analysis data of the complexes well confirmed their molecular formulae as shown in Table 1. Magnetic moments measurements, IR, UV-Vis and EPR spectroscopy achieved full structure characterization. The newly synthesized copper(II) complexes are green and brown, show remarkable stability under ambient conditions, and are freely soluble in methanol, DMSO, and DMF. Despite the numerous attempts that have been made, we have not been able to isolate a single-crystal of the current copper(II) complexes that would be suitable for structural studies by X-ray diffraction.

DMF solutions at a concentration of 10⁻³ M of the inspected copper(II) chelates exhibit electrolytic conductance at room temperature between 31.88 and 55.12 indicating their non electrolytic behavior (Geary, 1971). These results suggest the participation of the two counter anions (bromide ions) in the coordination sphere. The analytical results and the nonelectrolytic behavior support the specific molecular formulas shown in Table 1.

### 2. Experimental

The supplementary material (S1) contains the materials used, the physical measurements, and the practical details of oxidase biomimetic catalytic activity.

### 3. Results and discussion

#### 3.1. General

In situ, new tridentate pyridine-2,6-diimine (L1–4) as copper(II) chelates were prepared by template technique. In 50 ml absolute ethanol 0.024 mole of both 2,6-biacetyl pyridine and CuBr₂ were mixed and this mixture was stirred for one hour at ambient temperature to initiate the Schiff base condensation reaction. Then 0.048 mol of the respective aniline derivative was added and stirring was continued for another hour at room temperature as the colored products were formed. The products were separated by a filtration process, that followed by washing with alcohol, then ether. Finally, these colored precipitates were kept in a dry atmosphere in a desiccator over CaO for one week.

#### 3.2. Metal complexes synthesis by template technique

Attempts to prepare the current diimine ligands via direct interaction between 2,6-diaceetyl pyridine (2,6-DAP) and aniline derivatives were unsuccessful. Many Schiff condensation reactions depend on the presence of a metal ion that acts as a template (Lindoy, 1990; Ramadan, 2012a, 2012b; Ramadan & El-Emary, 1998). In some cases, the formation of Schiff

| Complex | Color | λM (Ω⁻¹ cm² mol⁻¹) | %C | %H | %N | %M |
|---------|-------|---------------------|----|----|----|----|
| 1. [CuL₁ Br₂] | Dark green | 55.12 | 39.62 | 2.79 | 11.31 | 9.89 |
| 2. [CuL₂Br₂].H₂O | Dark green | 43.76 | 35.67 | 2.18 | 6.52 | 9.11 |
| 3. [CuL₃ Br₂] | Black | 44.56 | 44.50 | 3.15 | 7.21 | 11.05 |
| 4. [CuL₄ Br₂] H₂O | Brown | 36.76 | 48.53 | 3.80 | 7.12 | 11.89 |

Table 1. Molecular formulae, physical properties and elemental analytical data of copper(II) complexes 1–4.
bases was only possible if there was a metal ion directing the steric path of the synthesis process. In this context, template technique is a successful for the synthesis of many organic ligands, which are generally isolated in the form of their metal complexes (Nivorozhkin et al., 1996; Thompson & Busch, 1964). In general, template technique can operate through kinetic and thermodynamic effects. The kinetic effect, in particular, provides pathways for desired products that can only be obtained if there is a metal ion while the thermodynamic effect includes the extraction of the desired product from equilibrium by stabilizing its metal compound (Cutler et al., 1985; Eggleston & Jackels, 1980; Lindoy, 1990; Nivorozhkin et al., 1996; Ramadan, 2012a, 2012b; Ramadan & El-Emary, 1998; Thompson & Busch, 1964; Welsh et al., 1977).

In the same regard, the template condensation reaction of 2,6-DAP and aniline derivatives was proceed in presence of copper(II) ion. The initiation step involves coordination of 2,6-DAP to copper(II) via the carbonyl oxygen which results in an activation of the carbonyl carbon for the nucleophilic attack of the nitrogen atom in the aniline. Intramolecular Schiff condensation with removal of H₂O results in the formation of azomethine bond coordinated with the copper(II) ion through nitrogen (Scheme 1). This procedure may be repeated twice until the coordinated diimine ligand is formed. For this multi-step path, the thermodynamic effect will operate in all steps while the kinetic effect controls the final step. The potential interaction steps are detailed in Scheme 1.

![Scheme 1. Temple synthesis of copper(II) complexes 1-4](image)

**Table 2. Thermogravimetric analysis degradation of copper(II) complexes 1-4.**

| Complex | Temperature °C | DTGmax | % Mass loss | Species formed |
|---------|----------------|--------|-------------|----------------|
| 1. [CuL₁ Br₂] | 180-200 200 | 13.54(12.76) | CuL Br | |
| 200-280 250, 270 | 32.12(32.06) | CuL(0.7) |
| 280-1020 600, 890 | 42.11(41.70) | CuO |
| 2. [CuL₂Br₂].H₂O | 60-120 105 | 2.15(2.04) | CuL Br₂ |
| 190-240 230 | 22.25(22.45) | CuL |
| 240-940 300, 400, 600 | 64.45(63.88) | CuO |
| 3. [CuL₃ Br₂] | 180-200 190 | 14.05(14.07) | [[CuLBr] |
| 200-300 290 | 26.09(26.20) | CuL(0.6) |
| 300-1000 600, 840 | 45.67(45.75) | CuO |
| 4. [CuL₄ Br₂].H₂O | 60-105 75 | 3.68(3.43) | CuL Br₂ |
| 210-260 240 | 14.00(13.73) | CuLBr |
| 260-425 280, 420 | 37.05(37.14) | CuL(0.6) |
| 650-1100 700 | 31.63(32.09) | CuO |

**Table 3. IR spectra (cm⁻¹) of copper(II) complexes 1-4.**

| Complex | ν(H₂O) | ν(C = N) | ν(py) | δ(py) | ν(N-Cu) | ν(Cu-Br) |
|---------|-------|---------|-------|-------|--------|---------|
| 1. [CuL₁ Br₂] | – | 1589 | 1479 | 651 | 501 | 410 |
| 2. [CuL₂Br₂].H₂O | 3448 | 1589 | 1481 | 678 | 505 | 415 |
| 3. [CuL₃ Br₂] | – | 1583 | 1467 | 686 | 449 | 420 |
| 4. [CuL₄ Br₂].H₂O | 3856 | 1592 | 1486 | 678 | 570 | 420 |
3.3. Thermal analysis

The analytical data indicated that, two of the present complexes contained water and thus a thermogravimetric analysis (TGA) was performed to elucidate the state of this hydration content. All thermal decomposition measurements were carried out under the influence of nitrogen, from room temperature to 1100 °C and the results obtained are shown in the Table 2 while the corresponding thermograms are given in supplementary materials S2–S5.

The anhydrous complexes 1 and 3 show a similar pyrolysis patterns that persists in three phases in the thermal range from room temperature to 1100 °C. The initial thermal mass loss phase refers to the volatilization of a bromine ligand bound to the metal ion. An explanation of the nature of volatile fractions at this stage finds support from compatibility between practical (13.54 and 14.05%) and theoretical (12.76 and 14.07%) values of mass loss. Since the onset of mass loss at this stage occurs above 190 °C, therefore any water content present in these metal chelates has been excluded.

The second phase of mass loss shows that the resulting intermediates decompose further in the temperature range of 200–300 °C, with DTGmax peaks at 290, 250 and 270 °C as shown in Table 2. This step involves removing the remaining coordinated bromide ion as well as partial pyrolysis of the chelated organic ligand.

The third stage of thermolysis, which occurs in the thermal range from 280 to 1020 °C with a DTGmax range of 600–890 °C, experiences the largest loss of mass, in successive steps ending with the formation of CuO.

For the hydrated complexes 2 and 4, the primary pyrolysis refers to surface water volatilization occurring between 60 and 120 °C with DTGmax peaks at 75 and 105 °C. For this stage, the range of values for the experimental mass loss (2.15 – 3.68%) corresponds to the range of theoretical values (2.04–3.43%). This easy volatilization of water molecules indicates the surface nature and excludes their presence in the coordination sphere.

The second thermal decomposition occurs between 190 and 260 °C with DTGmax at 230 and 240 °C indicates the complete volatilization of the bromo content for complex number 2 and removal of half of the coordinated bromide ions in the case of complex 4. The observed weight losses are 22.25 and 14.00% for complexes 2 and 4 respectively in accordance with calculated values of 22.45 and 13.73 (Table 2).

The final pyrolysis phase takes place in the thermal range of 240–1100 °C with DTGmax peaks within 280–700 °C that witness volatilization of the remaining bromine content for complex 4 and complete decomposition of the organic ligand for both 2 and 4. The residual weight losses are 0.85 and 0.10% for complexes 2 and 4 respectively in accordance with calculated values of 22.45 and 13.73 (Table 2).

3.4. FTIR spectra

To ensure the formation of Schiff bases in situ, as well as to identify the coordination pattern in the copper(II) complexes under study, infrared spectroscopy was used. In this regard, the FTIR spectra of the free components of Schiff bases were compared with those of the metal complexes formed by Schiff condensation in the presence of the copper(II) ion. The bands assignment data are tabulated in Table 3, while the corresponding charts are in the supplementary materials S6–S9. The spectra of copper(II) chelates showed the absence of distinct bands for the free primary amine which appeared at 3380, 3400 and 1610 cm⁻¹ (Table 3), in the spectra of free aniline derivatives. The same finding was noticed for the spectrum of the free 2,6-biacetyl pyridine where the characteristic band of the carbonyl group (C = O) appeared at 1700 cm⁻¹ was disappeared in the case of copper(II) complexes. This vanishing of the characteristic bands of both the primary amine and carbonyl group in the spectra of the prepared copper(II) complexes is a practical confirmation of the occurrence of Schiff condensation and formation of copper(II) dimines. In the same context, the appearance of the stretching frequencies characteristic to the azomethine linkage in the wavelength range of 1583–1592 cm⁻¹ in the spectra of CuII complexes, confirms what was previously deduced for the formation of Schiff bases as metal chelates (Nakamoto, 1986). The data in Table 3 show that the stretching frequencies of the complexed azomethine group is less than that normally appears for the free Schiff base linkage (Silverstein, 1981) which may be due to the driftage of the lone pair of the imine nitrogen towards the coordinated metal ion (Ilhan et al., 2007; Lopez-Garriga et al., 1986).

The observed displacement of the ν(C = N)pyridine band in the spectra of the copper(II) complexes compared to its position in the spectrum of the free 2,6-biacetyl pyridine indicates the participation of pyridine nitrogen in the coordination to the copper(II) ion (Table 3). In the same vein, the spectra of the CuII chelates exhibit medium bands at 1030 cm⁻¹ and 688 cm⁻¹ assignable to ν(Cu-N) and ν(Cu-Br) respectively in the case of copper(II) based complexes is conclusive evidence of the bonding pattern in these metal complexes (Temel et al., 2004).

As regards, the hydrated complexes, the absorption due to the lattice water is shown as a wide band at about 3400 cm⁻¹ (Table 3) which is attributable to ν(O-H). In the same respect, having a shoulder at 1630 cm⁻¹ which is the absorption frequency of the symmetric pattern of OH for the lattice water supports the above assignment at 3400 cm⁻¹ (Nakamoto, 1986). These spectral results are in agreement with measurements of the thermal analysis, which confirmed the surface nature of the water content of these CuII complexes.

Based on the results of analytical and thermal analysis as well as spectroscopy investigations in addition to the electrolytic conductance data, the molecular formulae for current
metal chelates have been proposed as mentioned in Scheme 1.

3.5. Electronic absorption spectra

The distinguishing UV-Vis measurements of the inspected CuII imines were performed in DMF and the obtained results are presented in Table 4, while the spectral plots are illustrated in S10-S13. All the complexes exhibit similar spectral features, which involve strong absorption in the high energy region between 27,112 and 31,585 cm⁻¹ attributable to n → π⁺ transition (West et al., 1991, 1998). With respect to the present penta-coordinated copper(II) complexes the energy of n → π⁺ band originating from the azomethine linkage greatly depend on the electronic properties of the substituents of present tridentate Schiff–bases. In this regard, the electron-donating substituents increase the energy of λmax and vice versa in the case of the electron withdrawing groups. This dependence of the charge transfer energy for n → π⁺ on the electronic properties of the ligand substituents for the copper(II) based imines has been previously reported (West et al., 1995). In the same respect, the Lewis acidity of the CuII center shows the dependence on the frequency values of the n → π⁺ charge transfers arising from the imine linkage (C = N) (West et al., 1995). Accordingly, the Lewis acidity of the present copper(II) chelates follows the sequence: 1 > 2 > 3 > 4.

As shown in Table 4, the recorded wavenumber values in the visible region, 11,859–13,787, 15,273–16,493, and 16,732–17,968 cm⁻¹ can be ascribed to the characteristic d-d transitions of the penta-coordinated CuII nucleus in a square pyramidal (sq py) stereochemistry (Lever, 1984). These energy values may originate from the electronic transitions dx⁻²–y², dxy → dx²–y² and dz² → dx²–y² for the copper(II) core in analogy with other CuII-based complexes in sq py structure (El-Metwally et al., 2006; Onawumi et al., 2008; Patel et al., 2007). The incoming magnetic measurements specifically electron spin resonance; support strongly the suggested sq py geometry for the present penta coordinated CuII chelates.

The spectrum of the free 2,6-diacetethylpyridine in the UV-visible region reveals a strong absorption band at 40,000 cm⁻¹ arising from pyridine π → π⁺ transition (Selmeczi et al., 2003). Likewise, the running CuII imines exhibit a similar strong absorption in the range from 32,258 to 39,215 cm⁻¹ which by analogy, is due to pyridine π → π⁺ transition. This remarkable bathchromic shift in the spectra of the metal chelates strongly supports involvement of pyridine nitrogen in the metal chelate formation (Gang & Yuan, 1994; Ismail, 1997).

3.6. EPR spectra and magnetic moment measurements

The effective magnetic moments of the copper(II) chelates under study are in the range from 1.94 to 2.15 BM (Table 5) which refer to the monomeric nature of the complexes. These results indicate the magnetic dilution behavior of the present metal chelates and exclude any magnetic exchange interaction in the surrounding environment.

In the absence of the structural determination by X-ray for penta-coordinated CuII-based compounds, it would be very difficult to define stereochemistry. However, EPR spectra provide valuable information to solve this problem specifically if the ESR-spectra of the rhombic type. Fortunately, the EPR spectral patterns of the present CuII chelates are of the rhombic type, which helps to determine the geometric index R.

In this regard, measurements of the X-band ESR spectra were performed at room temperature for the polycrystalline samples with a frequency of 9.1 GHz under magnetic field strength 3100 gauss and scan rate 1000. The spectral parameters gav, gx, gy, gz, Gav, Gx, Gy, Gz and G were computed and recorded in Table 5 while the related plots are given in S14-S17.

Complexes 1, 2, 3 and 4 show almost similar spectral patterns involving three lines related to the g-values gav, gx, gy and gz.
where $g_x \neq g_y \neq g_z$. The geometrical quotient, $(R)$, was determined from the relation $R = (g_x - g_y)/(g_y - g_z)$ to distinguish between the square pyramidal and trigonal bipyramidal stereochemistries of the penta-coordinated copper(II) complexes (El-Shazly et al., 1980). For $R > 1$ the stereochemistry is trigonal bipyramidal and the ground state is $dz^2$ (Chandra & Gupta, 2005a; Sacconi, 1968). On the other hand, when $R$ less than one the related geometry is square pyramidal and thus the unpaired electron of $d^9$ system is in the nonbonding orbital $dx^2-y^2$ (Barbucci et al., 1977; Chandra & Gupta, 2005b). The computed values of $R$ for complexes 1, 2, 3 and 4 are 0.938, 0.985, 0.793 and 0.6754 respectively less than one indicating the square pyramidal stereochemistry in agreement with results of the previously mentioned electronic absorption spectra.

The following relations were used to determine $g \perp$, $g_{av}$ and $G$ while $g_{jj}$ is directly equals to $g_{av}$, $g \perp = 1/2(g_y + g_z)$, $g_{av} = 1/3(g_{jj} + 2g \perp)$ and $G = (g_{jj} - 2)/(g \perp - 2)$ (Hathaway & Billing, 1970). The calculated values of $g_{av}$ are greater than 2.0023 (Table 5) suggesting that the covalent bonding pattern is predominant between the copper(II) core and the donor sites of the coordinated ligands system (Fidone & Stevens, 1959). In addition, the calculated $G^-$ values are greater than four, which excludes any spin interaction between the adjacent metal centers in the solid state and confirms what has been demonstrated based on the magnetic moment values mentioned above (Hathaway et al., 1969).

### 3.7. Geometrical optimization

Due to the practical difficulty in obtaining a suitable crystal of the copper(II) complexes under study, we resorted to the computational studies to verify the final structures of these metal chelates. In this context, theoretical studies are directed towards identifying molecular geometric shapes that describe the nature of chemical bonding and the reactivity of chemical species. However, DFT technique (Calais, 1993) has been applied to optimize the structure of the studied diimines and their copper(II) chelates. Beck’s three parameter model along with the Lee–Yang–Parr functional (B3LYP) (Becke, 1993; Lee et al., 1988) are employed. The stereochemical parameters of the studied ligands were calculated and optimized at the B3LYP/6-311g while the geometric parameters of the corresponding copper(II) complexes were calculated and optimized at the B3LYP/LanL2DZ level. All the calculations carried out by Gaussian 09 W package (Frisch & Clemente, 2009). The optimized structure of diimine ligands 1, 2, 3 and 4 and their copper (II) complexes are shown in Figure 1 and S18-S20. Selected geometrical parameters such as bond lengths and angles in addition to the dihedral angles of the ligands and their copper(II) complexes are tabulated in Tables 6 and 7 in addition to S21-S23.

The data in the Tables 6 and 7 in addition to S21-S23 show that for the free ligands, the bond lengths of the two imine groups (C=N) specifically N(12)-C(10), N(13)-C(11) are slightly shorter compared with the corresponding copper(II) chelates. This result demonstrates that the bonding takes place through the nitrogen atoms of the Schiff base linkage. Accordingly, the nitrogen binding to the Cu$^\text{II}$ nucleus results in a weakening of the C=N bond, and hence the C=N bond length becomes longer in all complexes (Parr & Yang, 1989).

For the pyridine ring, the N(6)-C(1) and N(6)-C(5) bond lengths became slightly shorter for all Cu$^\text{II}$ chelates compared to those of the free ligands. This fact confirms the participation of nitrogen of pyridine in the coordination chromophore. In the same context, upon chelation of the diimine...
ligands to copper(II) ion, the bond angles and dihedral angles of the Schiff base linkage are somewhat altered (see Tables 6, 7 and S21–S23).

For the five coordinated metal complexes, it is difficult to determine directly the spatial shape as is the case for the present penta coordinated CuII-based chelates. However, the angular structural parameter $\tau_5$ is helpful for distinguishing between the expected stereochemistries trigonal bipyramidal and square pyramidal. Therefore, the geometric parameter $\tau_5$ was computed from the relation: $\tau_5 = (\beta - \alpha)/60$; where $\alpha$ and $\beta$ are the largest angles around the metal core. For the ideal square pyramidal stereochemistry, the computed value of $\tau_5$ is zero whereas in the case of the trigonal bipyramidal geometry in a perfect state the corresponding $\tau_5$ value is one (Thorn & Sheldrick, 2013). For complexes 1, 2, 3 and 4 the computed values of $\tau_5$ (Table 6) are 0.0358, 0.063, 0.0935 and 0.0980 respectively indicating distorted square pyramidal polyhedron. For the present penta coordinated geometries, the specific equatorial donor sites are N(12), N(13), Br(45), and Br(46). It is observed that the two bromide ions slightly bent away from copper(II) ion resulting in the angle values of Br(45)–Cu(44)–Br(46) to be in the range from 148.261° to 150.865° (Table 6). In the same context the apical position of the square pyramidal geometry is occupied by the pyridine nitrogen N(6).

Notably, the results of geometrical optimization based on DFT treatments are consistent with the interpretations of the spectroscopic measurements discussed early and confirm the final structure of the current CuII-based chelates.

### 3.8. Electrochemical properties

The redox behavior of current copper(II) imines was investigated, since their redox potential is an intrinsic parameter of their performance as an oxidase mimics. The redox potentials of these oxidase functional models and the intermediates generate during the catalytic cycle must match some criteria. Accordingly, electrochemical measurements were conducted to characterize the impact of the ligand substituents on the CuII/CuI behavior of these oxidase mimics. In this regard, the cyclic voltammetric measurements were performed in CH3OH and in presence of NBu4PF6 (0.1 M) as supporting electrolyte. The resulting redox waves were observed between −1.0 and 1.5 V versus Ag/AgCl at potential scan rate of 100 mV s$^{-1}$. All complexes exhibit redox behavior as shown in the supplementary materials S24–S27 characterized by quasi-reversible patterns for one electron transfer processes between copper(II) and copper(I). The anodic peak recorded as a result of reduction of CuII to CuI appears within the voltage range from 315 to 375 mV (Table 8) followed by the emergence of an irreversible anodic peak at about −500 mV attributable to the reduction of the chelated imine ligand. On the other hand, the corresponding cathodic peak due to the reversible oxidation process of CuI to CuII occurs within the voltage range of 375–436 mV.

The computed redox potential $E_{1/2}$ values are 345, 405, 398, and 357 for complexes 1, 2, 3 and 4 respectively which can be compared to similar copper(II) complexes (Ramadan et al., 2019, 2020b). In the same respect, the specified $\Delta$E values (Table 8) closely coincide with the Nernst value (60 mV) indicating a single electron transfer in a reversible process for the couple CuII/CuI.

The values of the corresponding current ratio ($I_{pc}/I_{pa}$) are close to the unit confirming the reversible electrochemical behavior of the present copper(II) chelates (Karlin et al., 1980). This observed electrochemical conduct indicates the ability of current ligands to fine-tune the reversible one electron transfer process and is a fundamental requirement for these copper(II) complexes to perform their tasks as oxidase mimics. In view of this, the electrochemical behavior of these redox centers can be described as in Scheme 2 in accordance with similar Schemes for related copper(II) chelates.

### Table 6. Selected bond angles (°) and geometrical index ($\tau_5$) of complexes 1, 2, 3 and 4.

| Type          | [CuI,Br2] | [CuII,Br2] | [CuIV,Br2] | [CuV,Br2] | Type          | [CuI,Br2] |
|---------------|-----------|------------|------------|-----------|---------------|-----------|
| N6–Cu42–Br43 | $\tau_5 = 0.0358$ | $\tau_5 = 0.0935$ | $\tau_5 = 0.0980$ | $\tau_5 = 0.063$ | N6–Cu44–Br45 | 105.41  |
| N6–Cu42–Br44 | 103.71    | 102.70    | 101.78     | 103.26    | N6–Cu44–Br46 | 103.26    |
| N12–Cu42–N13 | 153.01    | 154.30    | 154.14     | 153.74    | N12–Cu44–N13 | 153.74    |
| N12–Cu42–Br43 | 93.17     | 92.91     | 93.24     | 92.61     | N12–Cu44–Br45 | 92.61     |
| N12–Cu42–Br44 | 93.56     | 93.96     | 93.76     | 94.11     | N13–Cu44–Br45 | 94.11     |
| N13–Cu42–Br44 | 93.17     | 93.17     | 93.24     | 92.61     | N13–Cu44–Br46 | 92.61     |
| Br43–Cu42–Br44 | 150.865   | 148.691   | 148.261   | 149.96    | Br44–Cu42–Br44 | 149.96   |

### Table 7. Selected bond distances (Å) around copper(II) ion of complexes 1, 2, 3 and 4.

| Type          | [CuI,Br2] | [CuII,Br2] | [CuIV,Br2] | [CuV,Br2] |
|---------------|-----------|------------|------------|-----------|
| Cu42–N6      | 2.085     | 2.059      | 2.061      | 2.068     |
| Cu42–N12     | 2.135     | 2.129      | 2.131      | 2.131     |
| Cu42–N13     | 2.135     | 2.129      | 2.132      | 2.131     |
| Cu42–Br43    | 2.504     | 2.510      | 2.504      | 2.505     |
| Cu42–Br44    | 2.511     | 2.552      | 2.556      | 2.540     |

### Table 8. Electrochemical data (mV) and (µA) of copper(II) complexes 1, 2, 3 and 4.

| Complex | $I_{pc}$ | $I_{pa}$ | $E_{1/2}$ | $\Delta E_p$ | $I_{pc}$ | $I_{pa}$ | $I_{pc}/I_{pa}$ |
|---------|----------|----------|-----------|--------------|----------|----------|-----------------|
| 1       | 375      | 315      | 345       | 60           | 4.2      | 3.8      | 1.10            |
| 2       | 436      | 375      | 405       | 61           | 5        | 4.5      | 1.11            |
| 3       | 428      | 368      | 398       | 60           | 5.1      | 4.3      | 1.18            |
| 4       | 388      | 326      | 357       | 62           | 2.8      | 2.4      | 1.16            |

*The characteristic peak of the coordinated imine ligand.
Several studies on biomimicking of catechol oxidase activity showed earlier that the binding of catechol to the central metal of the candidate oxidase model is a prerequisite for the initiation of the catalytic oxidation process (Kupan et al., 2009; Ramadan et al., 2019, 2012a, 2011, 2020a, 2020b, Shaban et al., 2012, 2019). In this context, the present square pyramidal copper(II) complexes have an empty coordination site that can receive the phenolate ion. Nevertheless, the binding of the phenolate ion to the CuII nucleus will lead to the formation of the hexa coordinated CuII chelate, which is unstable due to the Jahn Teller effect. Therefore, the initiation of the catalytic oxidation process will mainly depend on the occurrence of substitution reaction between the ligand and the copper(II) center.

**3.9. Kinetic study of ligand substitution**

Several studies on biomimicking of catechol oxidase activity showed earlier that the binding of catechol to the central metal of the candidate oxidase model is a prerequisite for the initiation of the catalytic oxidation process (Kupan et al., 2009; Ramadan et al., 2019, 2012a, 2011, 2020a, 2020b, Shaban et al., 2012, 2019). In this context, the present square pyramidal copper(II) complexes have an empty coordination site that can receive the phenolate ion. Nevertheless, the binding of the phenolate ion to the CuII nucleus will lead to the formation of the hexa coordinated CuII chelate, which is unstable due to the Jahn Teller effect. Therefore, the initiation of the catalytic oxidation process will mainly depend on the occurrence of substitution reaction between the ligand and the copper(II) center.

**Table 9. Ligand substitution data of copper(II) complexes 1, 2, 3 and 4.**

| Complex | Fast step $K_1$ | Slow step $K_2$ |
|--------|----------------|----------------|
| 1      | 57203          | 2858           |
| 2      | 1543           | 60.2           |
| 3      | 58.20          | 3.4            |
| 4      | 74.11          | 12.9           |

**Table 10. Rate constants and activation parameters for the reaction of copper(II) complexes 2 with TU in methanol.**

| Complex | $\Delta H^\circ$ [kJ mol$^{-1}$] | $\Delta S^\circ$ [J K$^{-1}$ mol$^{-1}$] | $\Delta H^\circ$ [kJ mol$^{-1}$] | $\Delta S^\circ$ [J K$^{-1}$ mol$^{-1}$] |
|---------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|
| 2       | 23.62±0.72                   | -190.48±2.4567                 | 27.148±2.3788                 | -201.15±8.11                   |

(Balamurugan et al., 2001; Flanagan et al., 1997; Kano et al., 1993; Kuchiyama et al., 1998; Martin et al., 1987; Meagher et al., 1992; Robandt et al., 1993; Shaban et al., 2009).
phenolate anion and the good leaving ligands. If the ligands attached to metal center are stronger nucleophiles than the studied phenolate ions, then an oxidation reaction will not take place. Therefore, the ability of current copper(II)-based complexes to adjust the easy substitution of the fifth ligand is the main factor for the initiation the catalytic oxidation processes of the studied phenols.

Accordingly, the present work includes a detailed study of the exchange reactions between the ligands attached to a copper(II) core and a strong nucleophile to verify the susceptibility of copper(II) chelates to substitute one of their coordinated ligands. Thiourea (TU) is one of the nucleophiles most used in the substitution reactions of metal complexes due to its large nucleophilicity, which prevents backward interaction with the departed ligand or solvent. Additionally, thiourea is a neutral nucleophile and thus does not affect the formation of the overall reaction charge in the transition state, which may include changes in the dipole moment (Shaban et al., 2012).

Since substitution reactions occur over a time scale of seconds, stopped-follow technique was used in the current study. Monitoring of these substitution reactions were performed within the spectral range of 300 to 600 nm, and a representative spectral profile is shown in Figures 2 and 3. The concerned reactions studied as a function of thiourea concentration and temperature and the results obtained are recorded in Tables 9 and 10.

The Methanolic solutions of TU were prepared at a concentration range of $1 \times 10^{-2}$ to $2.5 \times 10^{-2}$ M while the concentration of metal complex is 10 times lower than TU. At the recommended wavelength $\lambda = 425$ nm, the relationship (1) was adopted to fit the kinetic traces, absorbance versus time.

$$A = a_1e^{-k_{obsd1}t} + a_2e^{-k_{obsd2}t} + A_0$$ (1)

The kinetic conduct of the overall substitution process as shown in Figure 4 is distinctive to the biphasic pattern that includes two sequential substitution reactions 1 and 2 that involves replacement the nonelectrolytic bromo ligands by TU. The first reaction is fast while the second one is much slower and both are characterized by the second-order rate constants $k_1$ and $k_2$.

$$[\text{LCuBr}_2] + \text{TU} \xrightarrow{k_1} [\text{LCuTUBr}]^{+} + \text{Br}^{-}$$ (1)

$$[\text{LCuTUBr}]^{+} + \text{TU} \xrightarrow{k_2} [\text{LCu}(\text{TU})_2]^{++} + \text{Br}^{-}$$ (2)

In the absence of an adverse reaction, the overall substitution process reactions 1 and 2 results in the rate constants $k_{obsd1}$ and $k_{obsd2}$ being linearly dependent on TU concentration as given in relation 2:

$$k_{obsd1} = k_1[Nu] \quad \text{and} \quad k_{obsd2} = k_2[Nu]$$ (2)

As shown in the Table 9, the values of rate constant for the first ligand exchange reaction $k_1$ are greater compared to the second substitution reaction $k_2$. This finding attributed...
to three reasons; the first is steric hinderance effect of TU, which replaced the smaller bromo ligand. The second reason is the cationic charge of the copper(II) center whose influence has developed after replacing the negative bromo ligand with the neutralized thiourea molecule.

The third reason is due to the strength of the bonds between the copper(II) core and the donor atoms. Based on the bond distance, the current DFT study demonstrates that the bonds between the copper and bromo ions are the weakest compared to those between copper and nitrogen donors. As mentioned in Table 7, there is a difference in the bond lengths between the copper(II) ion and the two-bromo donors attached to it. Therefore, the overall substitution reaction proceeds in two phases where the rate constant for the first phase $K_1$ is greater than $K_2$ for the second phase depending on the bond length.

Looking at Scheme 1, we notice that the current copper(II) complexes have the same co-ligands but there are different substituents on the phenyl ring of Schiff base. Therefore, the first and second rates of substitution reactions are influenced by the electron density on the copper(II) center, which depends to a large extent on the electron withdrawing or donating properties of these substituents. For complexes 3 and 4, the presence of the electron donation groups, CH$_3$ and OH, increases the electronic density on the copper(II) center and thus reduces Lewis acidity resulting in a reduction of the substitution rates of $K_{obsd.1}$ and $K_{obsd.2}$ as shown in the Table 9. Conversely, complexes 1 and 2 contain in their structure the electron withdrawing groups, Br and NO$_2$, which reduces the electronic density on the Cu$^{II}$-center and increases the Lewis acidity of this metallic center. This evidenced by the observed increase in the values of the substitution rate constants $K_{obsd.1}$ and $K_{obsd.2}$. Ligand substituents influence on the Lewis acidity of copper(II) center was earlier studied spectroscopically (section 3.5) and the present trend is consistent with the aforementioned conclusion.

The question now arises: What is the mechanism by which the present substitution reactions have been implemented? To answer this question, one can resort to the thermodynamic parameter $\Delta S^\ddagger$ as a good probe to designate the mechanism of the studied substitution process. In this context, the negative value of $\Delta S^\ddagger$ denotes the associative mechanism while the positive value of $\Delta S^\ddagger$ is intended for the unimolecular dissociation $S_N^a$ mechanism (Langford & Gray, 1966). For assigning the employed mechanism for the present study, a representative complex was chosen for this purpose. The effect of temperature on substitution rate constants was examined with the stopped-follow technique in the temperature range of 8 to 35 $^\circ$C and the related kinetic trace is shown in Figure 5. Results obtained are represented graphically in Figure 6 as linear plots over the studied temperature range where the thermodynamic parameters namely enthalpy ($\Delta H^\ddagger$) and entropy ($\Delta S^\ddagger$) were determined (Table 10). The negative values of $\Delta S^\ddagger$ are $-190.48 \pm 2.4567$ and $-201.15 \pm 8.11$ for the fast and slow steps respectively. The negative sign of $\Delta S^\ddagger$ support the associative (A) mechanism for both fast and slow steps instead of the associative interchange (I$_a$) mechanism (S. Y. Shaban et al., 2015).

### 3.10. Oxidase mimetic activity

Catecholase activity is the process by which copper oxidase enzymes such as catechol oxidase and tyrosinase catalyze the aerobic transformation of catechols e. g. 3,5-di-tert-butylcatechol (3,5-DTBC$\ddagger$) to the highly reactive and biologically significant 3,5-di-tert-butylquinone (3,5-DTBQ), see reaction 3.

$$\begin{align*}
\text{Catecholase} & \rightarrow \text{O}_{2} \\
3,5\text{-DTBC} & \rightarrow 3,5\text{-DTBQ} + 2\text{H}_{2}\text{O} 
\end{align*}$$

(3)

Another member of the redox proteins containing copper is the oligomeric multicopper phenoxazinone synthase whose catalytic function is the six electrons oxidation process of ortho aminophenols (o-APH$_3$) to produce the pharmaceutical component ortho amino-3H-phenoxazine-3-one (APX) (see reaction 4) (Bertini et al., 2007).

$$\begin{align*}
\text{Catecholase} & \rightarrow \text{O}_{2} \\
\text{OAP} + 3/2\text{O}_{2} & \rightarrow \text{APX} + \text{H}_{3}\text{C} 
\end{align*}$$

(4)

This enzyme is of vital importance due to its catalytic role in the bacterial synthesis of the natural antibiotic Actinomycin D.

A detailed catalytic study was conducted to verify the relationship between oxidase-like activity and the structural properties of the current oxidase enzymes. In this regard, the course of the catalytic reactions was studied by stopped-follow technique by following the characteristic absorption of the oxidation products. Figures 7 and 8 show a representative spectral profiles for the catalytic oxidation of both 3,5-DTBC$\ddagger$ and o-APH$_3$ to 3,5-DTBQ and APX respectively where the corresponding traces are recorded for the growth of
absorbance at 400 and 433 nm versus time. The analysis of the stopped-follow spectral data demonstrated a biphasic kinetic behavior characterized by a fast reaction involves binding the substrate followed by a slow reaction which involves the heart of the catalytic oxidase process as described in reactions 5 and 6. In the case of catechol, the fast step represents the binding of one mole of the bivalent catecholate anion (3,4-DTBC\(^2^-\)) with two moles of copper(II) complex (reaction 5) followed by the fast two electrons oxidation of catecholate ring accompanied by the reduction of Cu\(^{II}\) to Cu\(^{I}\).

\[
2[\text{LCu}^{II}\text{Br}_2] + 3,5-\text{DTBCH}_2 \xrightarrow{k_1} [\text{LCu}^{II}\text{Br}_2]_2 \xrightarrow{k_2} 3,5-\text{DTBQ} + \text{H}_2\text{O}_2
\]

Concerning o-APH\(_3\) the initial step comprises binding of the monovalent ortho aminophenolate anion (o-APH\(_2\)\_bar) to the catalyst by the stoichiometry ratio of 1:1 (catalyst/o-APH\(_2\)\_bar) resulting in rapid oxidation of one mole of o-APH\(_3\) with concomitant reduction of Cu\(^{II}\) to Cu\(^{I}\) followed by the second step which includes the actual catalytic process.

To verify the catalytic potential of the present metal chelates, the catalytic oxidation experiments of the studied substrates were performed in the absence of the catalyst and the yield of both 3,5-DTBQ and APX was very low and thus could be neglected.

3.10.1. Kinetic study

A kinetic study conducted to gain a deeper understanding of the catalytic behavior of the running Cu\(^{II}\) oxidase mimics. Kinetic and catalytic parameters such as \(k_1\), \(k_{-1}\), \(k_{\text{cat}}\), \(K_M\), \(V_{\text{max}}\), and the ratio \(k_{\text{cat}}/K_M\) were determined. A representative kinetic traces obtained by stopped-flow spectrophotometry monitored at 400 and 433 nm respectively for the two reaction steps for the aerobic oxidation of both 3,5-DTBCH\(_2\) and o-APH\(_3\) in presence of complex 1 in MeOH at 296 K (a) in the case of 3,5-DTBCH\(_2\) and (b) for o-APH\(_3\).

Plots in Figures 11, 12 and S31–S34 reveal that in the range of low concentrations of the studied substrates, the observed rate constants show a linear pattern indicating first-order behavior. At higher substrate concentrations, kinetic saturation behavior predominated as the rate of the catalytic reaction was not affected by the increase in substrate amount. Based on the observed kinetic behavior of current oxidation reactions, the Michael Menten model can be adopted to calculate catalytic activity evaluation parameters such as \(k_{\text{cat}}\), \(K_M\), \(V_{\text{max}}\) and the ratio \(k_{\text{cat}}/K_M\) (Table 11).

Looking on Tables 9 and 11 one can note that, for the reversible reaction 5 the values of equilibrium constant (\(K_1\)), that measure the binding affinity of 3,5-DTBCH\(_2\) towards catalyst, depend on the labile ability of the studied Cu\(^{II}\) complexes and follows the sequence 1 > 2 > 4 > 3. This order corresponds to the substitution rates constant values as shown in the Table 11 as well as with the Lewis acidity trend discussed earlier in Section 3.9. These results find further support from the \(K_M\) values in Table 11, which holds the
same order of $K_1$; it is well known that $K_M$ is a practical measure for the binding affinity of the substrate towards the active site of the catalyst (Granata et al., 2004). It has become prevalent to describe the efficiency of the catalyst using the ratio $k_{\text{cat}}/K_M$, and its calculated values in the Table 12 exhibit roughly the same order as in the case of $K_1$ and $K_M$ with respect to 3,5-DTBCH$_2$. Thus, the Lewis acidity of Cu$^{II}$ centers and the binding affinity of the current substrates towards the Cu$^{II}$ complexes control the catalytic efficiency of these catechol oxidase mimics.

It should be noted that the electronic properties of the substituents in the present ligands are a major factor in controlling the structural properties and catalytic potential of the studied functional catechol oxidase models. With regard to $\omega$-APH$_3$, there is no interchangeable correlation between the specified values of $K_1$ and the labile character or Lewis acidity of the Cu$^{II}$ chelates examined. As well, no trend in the values of the ratio $k_{\text{cat}}/K_M$ that can be correlated with the values of both $K_1$ and $K_M$ as in the case of catechol. However, the running oxidase mimics exhibit catecholase activity lowers than phenoxazinone synthase like activity. The superiority of the phenoxazinone synthase mimetic activity over the catecholase-like activity of the present oxidase mimics can be attributed to the thermodynamic causes discussed in the next section.

To assess the catalytic capacity of the copper(II) complexes under study, the catalytic activity of the natural enzyme (catechol oxidase from sweet potatoes) was measured under the same conditions as the current catalytic measurements were made. The results obtained are represented in Figure 13 and from this plot the values of both $V_{\text{max}}$ and $K_M$ were estimated which are 3.167 and 0.004679 respectively. The calculated values for $k_{\text{cat}}$ and $k_{\text{cat}}/K_M$ ratio are equal to 3177 (s$^{-1}$) and 67.89 $\times$ 10$^4$ (mM$^{-1}$ s$^{-1}$) respectively indicating the high superiority of the natural enzyme. In the same respect, the catalytic potential of oxidase mimicry of current oxidase models is comparable to that mentioned in Table 12 for single-core Cu$^{II}$ chelates.

In the last decade, oxidase biomimetic catalytic activity has modeled by small molecular mass oxidase mimics and few Cu$^{II}$-based compounds have been reported to catalyze oxidation of both catechols and 2-aminophenols (Kupán et al., 2009; Ramadan et al., 2019, 2020a, 2020b, 2020c; Shaban et al., 2019). The ability of the catalyst to catalyze more than one type of catalytic process is the catalytic promiscuity. The present oxidase models show catalytic promiscuity because they can efficiently catalyze the aerobic oxidation of both 3,5-DTBCH$_2$ and $\omega$-APH$_3$. 

Figure 10. Absorbance-time traces for the reaction of complex 1 with 3,5-DTBCH$_2$ and $\omega$-APH$_3$ at $\lambda = 400$ and 433 nm respectively at room temperature; (a) for 3,5-DTBCH$_2$ and (b) in the case of $\omega$-APH$_3$.

Figure 11. $k_{\text{obs}}$ versus 3,5-DTBCH$_2$ concentration plot of reaction of 3,5-DTBCH$_2$ with complex 1 in CH$_3$OH at room temperature at $\lambda = 400$ nm. (a) is the fast step and (b) for the slow step.
3.10.2. Electrochemical, energetic and catalytic aspects

Cyclic voltammetry measurements were included in this work to verify the correlation of the electrochemical properties of the current copper-based complexes with their oxidase mimetic potential. The results in Table 8 indicate that the current copper(II) oxidase functional models can efficiently adopt a reversible single electron transfer process between Cu\(^{II}\) and Cu\(^{I}\) states which is a fundamental requirement for performing the catalytic oxidation cycle of the studied phenols. However, the redox potentials (Table 8) of the studied oxidase models are in the range of the standard potential of 180–370 mV of the blue copper proteins (Bertini et al., 2007).

Thermodynamic studies indicate that a direct interaction between molecular oxygen and organic materials is a favorable process but the rate at which proceed is very slow. The slowness of these reactions is attributed to the presence of \(O_2\) in the triple spin state \((S = 1)\) while organic substances are naturally present in the singlet state \((S = 0)\). This spin difference makes the interaction between \(O_2\) and the organic substance is kinetically unfavorable. The best way to overcome the kinetic obstacle of these reactions is to select an appropriate catalyst.

Several copper-based compounds have been ideally used to catalyze the oxidation of many phenols by activating the atmospheric oxygen (Bart et al., 2006; Knijnenburg et al., 2004; Ramadan et al., 2012, 2011; S. Shaban et al., 2012, 2019). In this respect, catalytic oxidation reactions emerge depending on the processes of electrons transfer from organic substrates to \(O_2\) through the metallic center of the

![Figure 12](image-url)  \(k_{\text{obs}}\) versus 3,5-DTBCH\(_2\) concentration plot for reaction of \(\alpha\)-APH\(_3\) with complex 1 in CH\(_3\)OH at room temperature at \(\lambda = 433\) nm. (a) represents the fast step and (b) is for the slow step.

![Figure 13](image-url)  \(k_{\text{obs}}\) versus 3,5-DTBCH\(_2\) concentration plot of reaction of 3,5-DTBCH\(_2\) with the natural enzyme (catechol oxidase from sweet potatoes) at room temperature at \(\lambda = 400\) nm.

### Table 11. Kinetic parameters of the catalytic aerobic oxidation of 3,5-TBCH\(_2\) and \(\alpha\)-APH\(_3\) by copper(II)-based complexes.

| Substrate | Complex | \(k_1\) (M\(^{-1}\) s\(^{-1}\)) | \(k_-\) (s\(^{-1}\)) | \(k_1/k_-\) (M\(^{-1}\) s\(^{-1}\)) | \(k_{\text{cat}}\) (s\(^{-1}\)) | \(V_{\text{max}}\) | \(K_M\) (M) | \(k_{\text{cat}}/K_M\) \times 10^3 (mM\(^{-1}\) s\(^{-1}\)) |
|-----------|---------|-----------------|----------------|-----------------|----------------|--------------|-----------|-----------------------------|
| 3,5-DTBCH\(_2\) | 1       | 12.31           | 12.31          | 12.31           | 12.31          | 7.096 \times 10^-3 | 8.258 \times 10^-3 | 3.43666 |
|            | 2       | 20.80           | 20.80          | 20.80           | 20.80          | 9.191 \times 10^-4 | 6.995 \times 10^-3 | 0.48634 |
|            | 3       | 68.80           | 68.80          | 68.80           | 68.80          | 1.123 \times 10^-3 | 6.04 \times 10^-3 | 0.07433 |
|            | 4       | 33.85           | 33.85          | 33.85           | 33.85          | 3.706 \times 10^-4 | 1.105 \times 10^-3 | 1.34117 |
| \(\alpha\)-APH\(_3\) | 1       | 1.170           | 1.170          | 1.170           | 1.170          | 2.926 \times 10^-3 | 3.352 \times 10^-3 | 3.4900  |
|            | 2       | 22.32           | 22.32          | 22.32           | 22.32          | 2.631 \times 10^-3 | 1.283 \times 10^-3 | 41.013  |
|            | 3       | 117.3           | 117.3          | 117.3           | 117.3          | 1.108 \times 10^-3 | 8.407 \times 10^-5 | 0.4390  |
|            | 4       | 2912            | 2912           | 2912            | 2912           | 9.773 \times 10^-4 | 8.885 \times 10^-5 | 36.813  |

### Table 12. Kinetic parameters of the catalytic aerobic oxidation of 3,5-TBCH\(_2\) and \(\alpha\)-APH\(_3\) by copper(II)-based complexes.

| Complex                  | Substrate  | \(k_{\text{cat}}\) (h\(^{-1}\)) | \(K_M\) (M) | \(k_{\text{cat}}/K_M\) \times 10^3 (M\(^{-1}\) h\(^{-1}\)) |
|--------------------------|------------|-------------------------------|-------------|----------------------------------|
| \([\text{Cu}LL]\) \([\text{NaClO}_4]\)_3 | 3,5-TBCH\(_2\) | 58.0 \times 10^{-4} | 2.416 \times 10^3 |
| \([\text{Cu}LL]\) \([\text{NaClO}_4]\)_3 | \(\alpha\)-APH\(_3\) | 50.0 \times 10^{-4} | 1.724 \times 10^3 |
| \([\text{Cu}([\text{Val})(bpy)]Br]\) | 3,5-TBCH\(_2\) | 126.0 \times 10^{-4} | 11.05 \times 10^3 |
| \([\text{Cu}([\text{Val})(Phen)]Br]\) | \(\alpha\)-APH\(_3\) | 360.0 \times 10^{-4} | 13.48 \times 10^3 |
| \([\text{Cu}([\text{Val})(bpy)]Br]\) | 3,5-TBCH\(_2\) | 366.0 \times 10^{-4} | 9.29 \times 10^3 |
| \([\text{Cu}([\text{Val})(bpy)]Br]\) | \(\alpha\)-APH\(_3\) | 360.0 \times 10^{-4} | 13.48 \times 10^3 |
| \([\text{Cu}([\text{Cl}])_3\] \([\text{Cl}])_4\] \([\text{H}_2\text{O}]\) | 4-TBCH\(_2\) | 64.80 \times 10^{-4} | 5.40 \times 10^3 |
| \([\text{Cu}([\text{Cl}])_3\] \([\text{Cl}])_4\] \([\text{O}]\) | \(\alpha\)-APH\(_3\) | 202.8 \times 10^{-4} | 15.60 \times 10^4 |

*Ramadan et al. (2020a); **Ramadan et al. (2019); ***Ramadan et al. (2020c).
catalyst. The running kinetic investigations showed that, binding of the studied substrates to Cu^{II} core is the spark for beginning the oxidation processes. Accordingly, the electron transfer processes take place by the inner sphere mechanism because copper(II) center will play the role of a bridge between the substrate and O_{2}. These electron transfer processes are pattern of the donor/acceptor interactions. In this case, the electrochemical information will be very helpful in the estimation of the drive force (\Delta G^o) of the studied bimolecular electron transfer reactions from the relation (Bertini et al., 2007):

$$\Delta G^o = -n \times \left[ E^o_{\text{acceptor}} - E^o_{\text{donor}} \right] \times 96.48 \text{kJ mol}^{-1} \text{V}^{-1}$$

However, the formation of the intermediate ternary complex [substrate/catalyst/O_{2}] is the percourser for the actual catalytic oxidation process. This finding was previously reported in similar studies (Kupán et al., 2009; Ramadan et al., 2012, 2011, 2020a, 2020b, 2020c, Shaban et al., 2012, 2019) and has been confirmed by the current stopped-follow kinetic investigations.

The negative sign of the drive force (Table 13) indicates that, the first electron transfer process during the current oxidation processes occur spontaneously. The drive forces associated with the oxidation of 3,5-DTBC fall in the range from -27.49 to -21.78 kcal mol\(^{-1}\) V\(^{-1}\) and are higher than those associated with oxidation of o-APH\(_3\) which lies between -51.61 and -45.82 kcal mol\(^{-1}\) V\(^{-1}\). The less free energy associated with catalytic chemical reactions, the more efficient the catalyst used and vice versa. The positive sign for \(\Delta G^o\) observed for the second electron transfer process during catechol oxidation also indicates that this process is thermodynamically relatively unfavorable (Valentine et al., 2012). These results could explain the higher phenoxazinone synthase like activity as compared to the catechol oxidase mimetic activity for the current copper(II) oxidase mimics. Overall, the negative sign of \(\Delta G^o\) indicates that the running oxidase models successfully drive the electrons transfer from the HOMO of the current substrates to the LUMO of O_{2} through copper(II) center.

### 3.10.3. Test of O_{2} participation

The running catalytic oxidation study as well as similar studies indicates that the involvement of O_{2} in the oxidation processes of the substrates under study is essential to conduct the catalytic oxidation reaction. Confirming participation of O_{2} in the ongoing catalytic oxidation processes, some experiments were conducted in an inert atmosphere of nitrogen gas. Ongoing kinetic investigations indicate that the studied catalytic oxidation processes proceed in two steps and that the second step is mainly dependent on participation of O_{2}. Therefore, in the absence of the atmospheric oxygen, we were unable to observe this step because the spectral growth of the characteristic band of the oxidation product did not appear as illustrated in Figure 14.

### 3.10.4. Detection of H_{2}O_{2}

It is of great importance to identify the by-product of the running catalytic processes in order to define a reaction mechanism closer to the truth. However, O_{2} participation in the catalytic aerobic oxidation reactions of the studied phenols seldom yields water and often results in the conversion of O_{2} to H_{2}O_{2} as a by-product. To ensure practically the conversion of oxygen to hydrogen peroxide at the conclusion of the studied catalytic cycles, a test was performed for the oxidation of the iodide ion to the iodate ion by the action of H_{2}O_{2} as previously recommended (Sengupta et al., 2018). Conversion of I\(_{3}\)\textsuperscript{-}bar to I\(_{5}\)\textsuperscript{-}bar by hydrogen peroxide was monitored spectrophotometrically at 353 nm, which is the characteristic wavelength of I\(_{5}\)\textsuperscript{-}bar as seen in Figure 15.

### Table 13. Electrochemical data (V) and free energy, \(\Delta G^o\) (kJ mol\(^{-1}\) V\(^{-1}\)) of the catalytic aerobic oxidation of 3,5-DTBC\(_2\) to 3,5-DTBQ and o-APH\(_3\) to APX.

| Complex | \(E^o_{\text{Cu}^{II}}\) (V) | \(\Delta G^o\) \text{Cu}^{II}/2\text{Cu}^{II} \text{–} \text{O}_{2}/\text{O}_{2}^\circ \text{–} \text{O}_{2}/\text{O}_{2}^\circ\) | \(\Delta G^o\) \text{O}_{2}/\text{O}_{2}^\circ\) | \(\Delta G^o\) \text{O}_{2}/\text{O}_{2}^\circ\) | \(\Delta G^o\) \text{Cu}^{II}/\text{Cu}^{II} | Substrate |
|---------|----------------|---------------------------------|----------------|----------------|-----------------|----------|
| 1       | 0.345          | -21.78                          | 65.12           | 6.17            | -45.82          | 3,5-DTBC\(_2\) |
| 2       | 0.405          | -27.49                          | 70.91           | 11.96           | -51.61          |          |
| 3       | 0.398          | -26.82                          | 70.23           | 11.28           | -50.94          |          |
| 4       | 0.357          | -22.86                          | 66.28           | 7.33            | -46.98          | o-APH\(_3\) |
| Substrate |                  |                                 |                |                |                 | 3,5-DTBC\(_2\) |

Figure 14. Representative kinetic trace obtained by stopped-flow instrument monitored at 400 nm for the reaction of complex 1 with 3,5-DTBC in the presence and absence of O_{2} in MeOH at 296 K.

Figure 15. The electronic spectrum of I\(_{3}\)\textsuperscript{-}bar at 353 nm, which was formed by the oxidation of I\(_{3}\)\textsuperscript{-}bar by the action of H_{2}O_{2} as was released as a by-product during the current catalytic oxidation of 3,5-DTBC\(_2\).
3.10.5. Potential mechanistic pathways

The current spectroscopic, kinetic and electrochemical measurements demonstrated the ping-pong mechanism of the running aerobic oxidation processes for each of the studied substrates. Accordingly, in the case of 3,5-DTBCH₂, the oxidation process proceeds in two consecutive steps of electron transfer. The first step involves transferring two electrons from the bound catechol ring to the CuII nucleus resulting in conversion of CuII to CuI with the release of o-quinone. In the next step, atmospheric O₂ binds quickly to the two CuI cores and the resulting ternary complex undergoes bi-intramolecular electrons transfer from copper(I) centers to O₂.

This electronic transfer leads to reduction of O₂ to the peroxide ion (O₂⁻) which must protonated before leaving the copper(II) centers. Further reorganizations of peroxocuppper(II) lead to formation and release of H₂O₂ with the return of the catalyst to its original active form. The potential catalytic cycle of the current oxidation process proceeds in four steps A, B, C and D as represented in Scheme 3.

With respect to o-APH₃, the first oxidation step involves transfer of an electron from the bound amino phenolate ring to copper(II) center. The electronic movement in this step leads to reduction of copper(II) to copper(I) with the formation of o-aminosemiquinone (o-APSQH₂). As in the case of catechol oxidation the atmospheric oxygen oxidizes copper(I) with the formation of o-APSQH₂-catalyst-O₂. On the way for closing the cycle of catalysis, the intermediate, o-APSQH₂-catalyst-O₂, performs successive internal rearrangements to produce the o-BQMI with the release of hydrogen peroxide as a by-product. It should be noted here that o-BQMI converts to APX through more non-catalytic reactions, as reported by previous studies (Kaizer et al., 2002; Zaki et al., 2000). The probable pathway for the running catalytic oxidation process includes the four steps A, B, C and D as shown in Scheme 4.

It is worth mention that earlier studies (Kaizer et al., 2002; Zaki et al., 2000) displayed that o-benzoquinone monoimine (o-BQMI) is the precursor to APX formation. Then o-BQMI converts to the final product o-amino-3H-phenoxazine-3-ones (APX) via further uncatalyzed reactions.
Four copper(II)-base chelates containing diimine ligands derived from 2,6-biacetyl pyridine and many aniline derivati- tives were synthesized via template technique. Square pyramidal structure was assigned for the synthesized copper(II) chelates based on spectral studies and DFT calculation. The Lewis acidity of the copper(II) center is shown depending on the electron withdrawing and donating properties of the ligand substituents and follows the order: 1 \( > 2 > 3 > 4 \). The results of the geometrical optimization based on DFT treatments are consistent with those determined from spectroscopic measurements and confirm the final structure of the present copper(II) complexes. Electrochemical conduct indicates the ability of current ligands to fine-tune the Cu\(^{II}/Cu^I\) redox potential. The study of the ligand substitution indicates that the TU-induced stearic factor, the formal charge on the copper (II) cores, and the bond lengths between the metal ion and the donor sites control the substitution reactions. Based on the negative sign of \( \Delta S^\circ \), the mechanism by which the ongoing substitution reactions have been implemented is the associative (A) mechanism. Catechol oxidase and phenoxazinone synthase mimetic activity of the current Cu\(^{II}\) complexes was studied. In this context, the Lewis acidity of Cu\(^{II}\) centers and the binding affinity of the current substrates towards the Cu\(^{II}\)-based complexes control the catalytic efficiency. The running oxidase models exhibit catalytic promiscuity because they can efficiently catalyze the aerobic oxidation of both 3,5-DTBC\(H_2\) and \(\alpha\)-APH\(3\). The positive sign for \( \Delta G^\circ \) observed for the second electron transfer process during catechol oxidation process explain the superiorlity of the phenoxazinone mimic activity over the catecholase-like activity. The nature of substituents in diimine ligands is a key factor for tuning the structural properties and catalytic potentials of the present copper(II) oxidase functional models.

**Disclosure statement**

The authors declare that there are no conflicts of interest regarding the publication of this research paper.

**Funding**

Taif University Researchers Supporting Project number (TURSP-2020/05), Taif University, Taif, Saudi Arabia.

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