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Biocatalytic Potential of Dinuclear Complexes Containing Imine and Oxime Groups: A Study on Catalase, Catecholase, and Phenoxazinone Synthase Enzymatic Activities

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ABSTRACT

The development of biomimetic catalysts has emerged as a promising alternative to natural enzymes, which often suffer from instability under operational conditions. In this study, six dinuclear metal(II) complexes containing imine and oxime groups, three homodinuclear Cu(II)-Cu(II) and three heterodinuclear Cu(II)-Mn(II), were evaluated for their catalase-, catecholase-, and phenoxazinone synthase-like activities. Catalase-like activity was assessed by measuring the volume of O2 evolved from the catalytic disproportionation of hydrogen peroxide. Catecholase- and phenoxazinone synthase-like activities were evaluated spectrophotometrically using 3,5-di-*tert*-butylcatechol and 2-aminophenol as model substrates, respectively. The catalytic reaction's rate constants (k_{obs}) were calculated to quantify the enzymatic behavior. The results revealed that homodinuclear Cu(II)-Cu(II) complexes exhibited significantly higher catalytic activity than their heterodinuclear counterparts across all three reactions. Complex 3 (*p*-acetylaniline-substituted) showed the highest catalase activity with 17 mL O2 evolution. In contrast, complex 2 (*p*-toluidine-substituted) demonstrated the highest catecholase- and phenoxazinone synthase -like activities with rate constants of 0.2385 min^{-1} and 0.2705 min^{-1}, respectively. The enhanced activity was strongly correlated with the electronic nature of the substituents: electron-donating groups promoted redox cycling, whereas electron-withdrawing groups modulated Lewis acidity at the metal center. This comprehensive study highlights the catalytic potential of diimine-dioxime-based dinuclear metal complexes. It emphasizes the impact of metal identity and ligand design on enzyme-mimicking functions, offering valuable insights for future applications in green chemistry, biotechnology, and medicine.

Key Words: *Metal complex, catalase, catecholase, phenoxazinone synthase*

1. INTRODUCTION

Enzymes are essential to life, driving countless biochemical reactions with incredible speed and precision. Despite this, their use outside of natural systems is often limited. They're fragile, expensive to produce, and typically degrade under non-biological conditions. These drawbacks have led researchers to look for synthetic alternatives that mimic enzymatic activity without the same constraints (1). One promising route involves designing metal-based compounds, particularly those built around redox-active transition metals, that can conduct similar reactions more reliably under a broader range of conditions (2,3).

Among the strategies explored, ligands containing imine and oxime groups have shown promise. These groups help form stable metal complexes, yielding structures that remain well-organized and participate in electron-transfer processes essential for catalysis (4). Dinuclear metal complexes, particularly those combining copper(II) and manganese(II), have attracted attention due to their functional resemblance to natural enzymes (5-10).

In this study, we tested the catalytic behavior of six different dinuclear metal(II) complexes using three biologically inspired model reactions: decomposing hydrogen peroxide, as in catalase; oxidizing catechols, as in catecholase; and promoting the oxidative coupling of 2-aminophenol, as in phenoxazinone synthase activity. Each process contributes to biological functions, ranging from antioxidant defense to neurotransmitter processing and natural product biosynthesis. Through kinetic studies and spectrophotometric analysis, we aimed to uncover how differences in metal pairing and ligand design influence performance. The primary objective extended beyond the mere replication of natural processes; it encompassed a deeper understanding of how to construct efficient, reliable catalysts for practical applications across diverse fields, including environmental chemistry, industrial synthesis, and medicine.

2. MATERIALS AND METHODS

2.1. Chemicals and Physical Measurements

All chemicals employed in this study were purchased from Merck and utilized without further purification. A PG T80+ spectrophotometer was used for all spectroscopic measurements.

2.2. Preparation of the Metal(II) Complexes

The compounds used in the enzymatic activity study were synthesized according to literature procedures (11,12). The molecular structures of the metal complexes employed in this work are shown in Figs. 1 and 2.

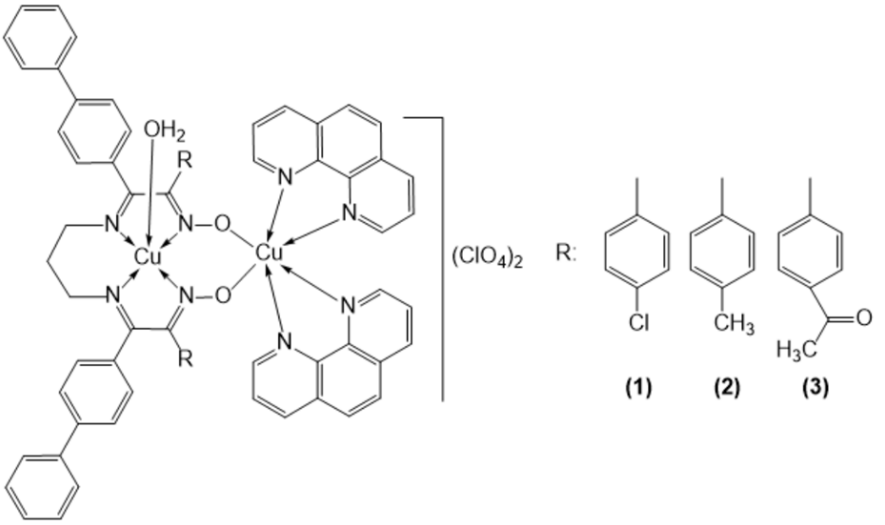


Figure 1. Molecular geometry of the homodinuclear Cu(II)-Cu(II) complexes

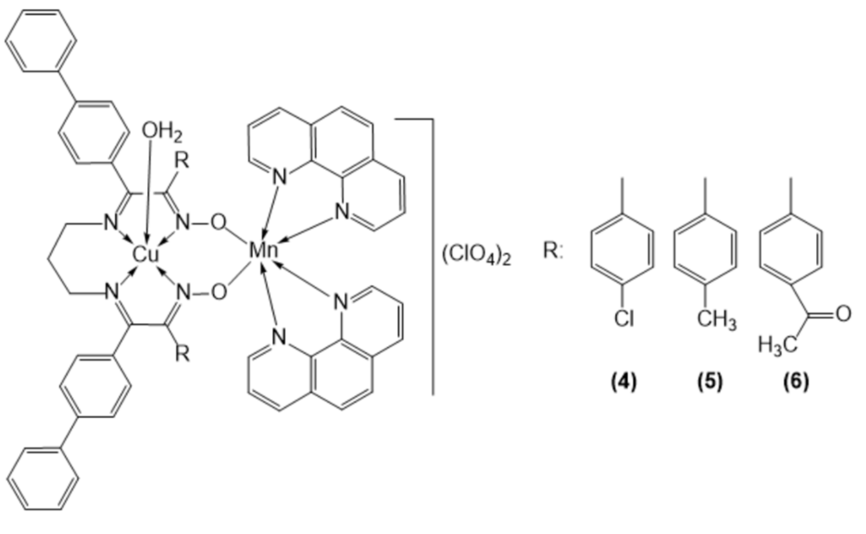


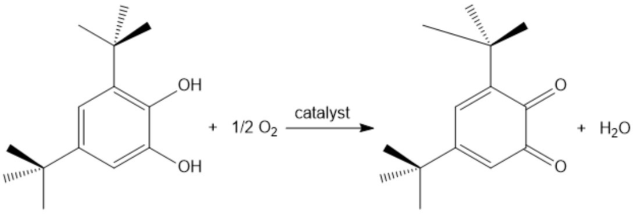
Figure 2. Molecular geometry of the heterodinuclear Cu(II)-Mn(II) complexes

2.3. Catalase-like activity

$$2H_2O_2 \xrightarrow{\text{catalyst}} 2H_2O + O_2$$

The catalase-like activities of the compounds were evaluated using a modified version of the method proposed by Kaizer *et al* (13). The volumetric evolution of molecular oxygen resulting from the disproportionation reaction of H_2O_2 in the presence of dinuclear metal(II) complexes was measured, for the volumetric determination of O_2 released during the reaction of the metal(II) complexes with H_2O_2, 0.005 mmol of the metal(II) complex was weighed, dissolved in N,N-dimethylformamide, and transferred into a three-necked flask placed in a water bath at room temperature. One neck of the flask was connected to a water-filled burette, while the other necks were sealed with rubber septa. As a catalyst, 50 mg of 1-methylimidazole was added to the reaction medium. While the solution was stirred at room temperature, 1.33 mmol (0.150 mL) of H_2O_2 was injected through the rubber septum. The volume of O_2 gas evolved was measured at 60-second intervals.

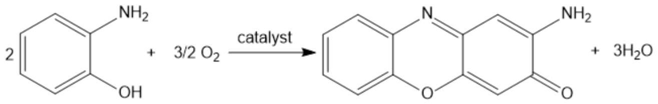
2.4. Catecholase-like activity



The catecholase-like activities of the compounds were investigated using slight modifications of previously reported procedures (14). Each complex was dissolved in methanol to prepare 1×10^{-4} M solutions for the assay. A 5×10^{-3} M solution of 3,5-di-*tert*-butylcatechol (3,5-DTBC), used as the substrate, was also prepared in methanol to achieve a 50-fold molar excess. Equal volumes (1:1) of the complex and substrate solutions were mixed and transferred into a quartz cuvette for spectrophotometric analysis. The reactions were monitored using a UV-vis spectrophotometer by recording time-dependent absorbance at specific wavelengths at 60-second intervals against a blank. An increase in absorbance at 400 nm was observed, characteristic of the formation of 3,5-di-*tert*-butyl-*o*-benzoquinone (3,5-DTBQ), the substrate's oxidation product. The observed rate constants (k_{obs}) for the formation of 3,5-DTBQ by the metal(II) complexes were determined using equation (1). In this equation, A_∞ and A_t denote the absorbance of the formed 3,5-DTBQ at infinite time and at time t, respectively.

$$\log(A_\infty/A_\infty-A_t)=(k_{obs})_1 t \qquad \text{eq (1)}$$

2.5. Phenoxazinone synthase-like activity



The reaction between 2-aminophenol (OAPH) and molecular oxygen was carried out in the presence of a catalytic amount of dinuclear metal(II) complexes (15). A 1.67×10^{-4} M solution of the complex in methanol was mixed with a 12.5×10^{-3} M solution of OAPH, used as the substrate. The resulting mixture was transferred into a quartz cuvette, and the reaction was monitored using a UV-vis spectrophotometer. Spectral scans were recorded at 60-second intervals. The oxidation of OAPH was monitored by measuring the increase in absorbance at 433 nm, a characteristic of the formation of 2-aminophenoxazin-3-one (APX), the oxidative coupling product. The observed spectral changes confirmed the phenoxazinone synthase-like catalytic activity of the complexes. The observed rate constants (k_{obs}) for the formation of APX by the metal(II) complexes were determined using equation (2). In this equation, A_∞ and A_t denote the absorbance of the formed APX at infinite time and at time t, respectively.

$$\log(A_\infty/A_\infty-A_t)=(k_{obs})_2 t \qquad \text{eq (2)}$$

3. RESULT AND DISCUSSION

3.1. Catalase-like Activity

This study evaluated the catalase-like activities of six different diimine-dioxime metal(II) complexes. The volume (mL) of O_2 gas released at various time points was measured for each complex. Catalase-like

Table 1. Catalase-like activity results of the complexes

	Volume of Evolved O_2 gas (mL)					
Time (min)	**1**	**2**	**3**	**4**	**5**	**6**
0	0	0	0	0	0	0
1	1.7	1	2	0.8	0.3	1
2	2.9	2	4	1.2	0.7	1.6
3	4.1	2.8	6.4	1.9	1.1	2.4
4	4.9	3.6	8.2	2.4	1.6	3.1
5	5.9	4.4	9.7	2.9	2.1	4
6	6.8	5.3	11.7	3.5	2.7	4.8
7	8	6.2	13.3	4	3.2	5.4
8	9.4	7	15	4.8	3.6	6.1
9	10.7	7.9	16.5	5.4	4.15	6.7
10	11.6	8.8	17	5.8	4.65	7.2
11	12	9.4	17	6.3	5.15	7.7
12	12.2	9.9	17	6.7	5.65	8.2
13	12.2	10.3	17	6.8	5.8	9
14	12.2	10.3	17	6.8	5.8	9
15	12.2	10.3	17	6.8	5.8	9

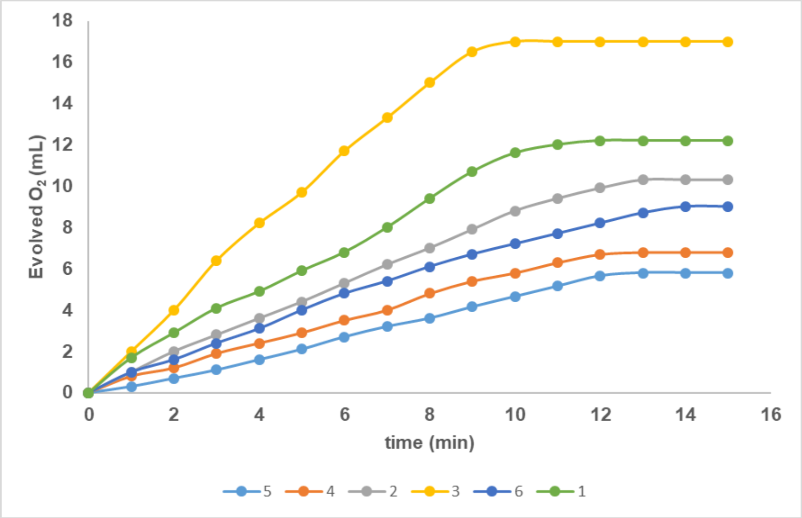


Figure 3. Graph of O_2 gas evolved by complexes over time

activity was assessed by measuring the amount of O_2 released from the disproportionation of hydrogen peroxide (H_2O_2), and the results are presented in Table 1 and Figure 3.

According to the results obtained, the activities of the complexes varied with the metal(II) ions they contained and the substituents. Homodinuclear Cu(II)-Cu(II) complexes generally exhibited higher catalase-like activity than heterodinuclear complexes. The most striking example in this group was complex 3 (*p*-acetylaniline derivative), which showed the highest catalase-like activity, producing 17 mL of O2 in 10 min. This value represented the highest activity in its group and among all complexes. Complex 2 (*p*-toluidine substituted) produced approximately 10.3 mL of O2 and exhibited moderate activity. Complex 1 (*p*-chloroaniline derivative) showed a kinetically slower and lower volume of O2 release, reaching only 12.2 mL.

All heterodinuclear complexes showed lower catalase-like activity than their homodinuclear counterparts carrying the same substituent group. For example, complex 6 (*p*-acetylaniline derivative) produced only 9 mL of O_2 in 15 min, indicating approximately 47% lower activity than complex 3. Similarly, complex 5 (*p*-toluidine substituted) reached the lowest O_2 production values with 5.8 mL, followed by complex 4 (*p*-chloroaniline substituted) with 6.8 mL. Based on these findings, it was concluded that Mn(II) ions limited catalase-like activity, likely disrupting the catalytic coordination of Cu(II) at the active site and preventing effective disproportionation of H_2O_2.

The substituents in the complexes' structures directly affected catalytic activity. The acetyl moiety was an electron-withdrawing functional group containing carbonyl (C=O). This feature could increase the Lewis acidic character of the Cu(II) center by decreasing the ligand's electronic density, thereby accelerating its interaction with H_2O_2. As a result, both complex 3 and complex 6 produced the highest O_2 yields in their series. In the *p*-toluidine group, the methyl ($-CH_3$) moiety became an electron donor, adding electron density to the aromatic ring. This situation could limit the reactivity by decreasing the electrophilicity of the Cu(II) center. Complexes 2 and 5 showed moderate activity due to this effect. In the *p*-chloroaniline group, although the chlorine atom has an electron-withdrawing character, it could also cause steric hindrance. In addition, electron withdrawal toward the Cu(II) center could excessively stabilize the ligation environment and reduce the interaction with H_2O_2. As a result, complexes 1 and 4 exhibited the lowest activity in both groups. As a result, homodinuclear Cu(II) complexes showed higher catalase activity compared to heterodinuclear Cu(II)-Mn(II) complexes. This indicated that Cu(II) centers were more catalytically active, and the presence of Mn(II) could limit the activity. In addition, the superior catalytic performance of complexes containing *p*-acetylaniline as a functional group, particularly complex 3, suggests that electron-withdrawing substituents may enhance catalase-like activity.

3.2. Catecholase-like Activity

Catecholase-like activity indicated the ability of metal complexes to oxidize substrates such as *o*-diphenols (e.g., catechol) to *o*-quinone products, and this activity was generally evaluated via the rate constant (k_{obs}, min^{-1}). In this study, the kinetic method investigated the catecholase-like activities of diimine-dioxime complexes 1-6, and the reaction rate constants were determined. Table 2 and Figure 5 show that complex 2 exhibited the highest catecholase-like activity, being the most reactive among the complexes, with a rate constant of 0.2385 min^{-1}. The lowest activity was observed in complex 6, with a rate constant of only 0.0744 min^{-1}. The spectrum scan performed in the presence of complex 2 is shown in Figure 4.

Homodinuclear Cu(II)-Cu(II) complexes 1-3 generally had higher rate constant values than heterodinuclear Cu(II)-Mn(II) complexes 4-6. This indicated that Cu(II) centers were more effective than Mn(II) in redox-

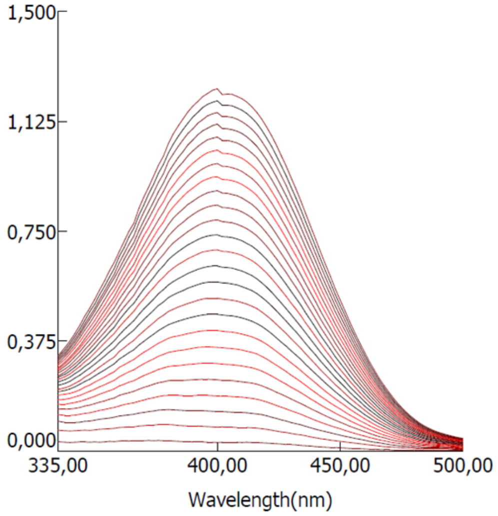


Figure 4. Spectral scans of the 3,5-DTBC to 3,5-DTBQ oxidation reaction catalyzed by complex 2, recorded at 60-second intervals

based reactions, such as catechol oxidation. Especially complex 2 (*p*-toluidine derivative) reached the highest rate, proving that Cu(II)-Cu(II) systems were quite effective in terms of catecholase-like activities. Lower rate constant values generally characterize heterodinuclear complexes. This decrease was likely due to the lower redox potential of the Mn(II) center relative to that of Cu(II), which limited the complex's electron-transfer capacity. The heterodinuclear complex with the highest rate constant was complex 5 (0.1255 min^{-1}), containing a *p*-toluidine substituent. Functional groups significantly shaped catalytic activity by modulating the complex's electronic properties. *p*-Toluidine, containing an electron-donating methyl group, facilitated the participation of the Cu(II) center in the redox cycle, resulting in the highest rate constants in complexes 2 and 5. *p*-Chloroaniline, carrying an electron-withdrawing chlorine group, stabilized the structure of the complex but reduced the reactivity, leading to moderate activity in complexes 1 and 4. *p*-Acetylaniline, with a strong electron-withdrawing acetyl group, restricted the redox flexibility, resulting in the lowest rate constants in complexes 3 and 6. The catecholase-like activities of the complexes were significantly affected by both the nature of the metal centers and the electronic effects of the substituent groups on the ligand. Homodinuclear Cu (II) complexes generally showed higher reactivity, while electron-donating substituent groups (especially *p*-toluidine) increased the catecholase-like activity. These results revealed that metal selection and ligand modification play decisive roles in catalyst design.

3.3. Phenoxazinone Synthase-like Activity

Phenoxazinone synthase activity has been a key criterion for modeling enzymes that catalyze the formation of phenoxazinone derivatives via the

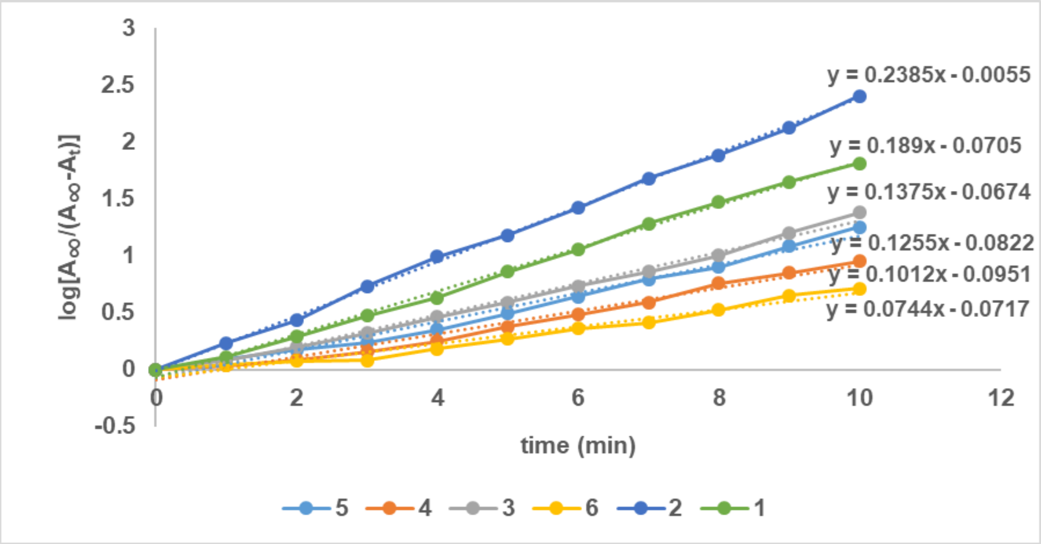


Figure 5. Comparison of observed rate constants for the catecholase-like activity of the complexes

Table 2. Observed rate constants (k_{obs}) for the oxidation of 3,5-DTBC to 3,5-DTBQ catalyzed by metal complexes

Compounds	Rate constant, k_{obs} (min^{-1})
1	0.1890
2	0.2385
3	0.1375
4	0.1012
5	0.1255
6	0.0744

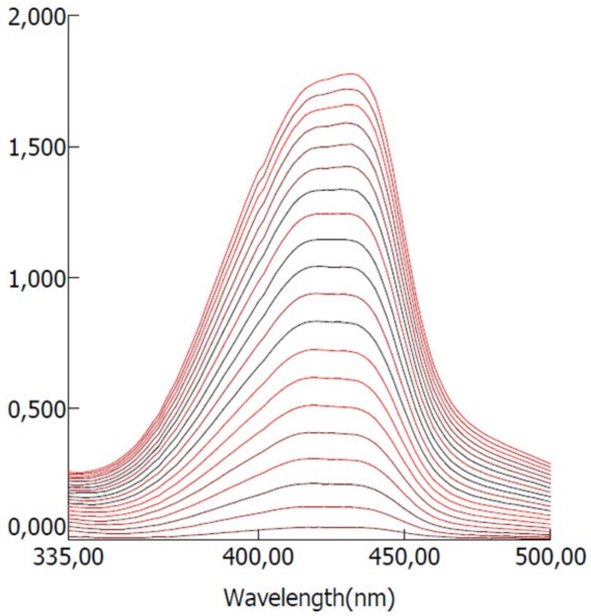


Figure 6. Spectral scans of the OAPH to APX oxidation reaction catalyzed by complex 2, recorded at 60-second intervals

oxidative dimerization of substrates such as 2-aminophenol. In this study, phenoxazinone synthase-like activities of homodinuclear Cu(II)-Cu(II) and heterodinuclear Cu(II)-Mn(II) metal complexes were investigated, and reaction rate constant (k_{obs}, min^{-1}) values were calculated. The obtained data are presented in Table 3 and Figure 7. The spectrum scan performed in the presence of complex 2 is shown in Figure 6. In the order made according to the rate constants, the highest phenoxazinone synthase-like activity was observed in complex 2 (0.2705 min^{-1}). This complex had a dinuclear Cu(II)-Cu(II) structure and a *p*-toluidine substituent group. It was followed by complex 3 (0.1923 min^{-1}) and complex 1 (0.1691 min^{-1}). The common feature of these three complexes is that they contain homodinuclear Cu(II)-Cu(II) centers, and it has been observed that these structures catalyze the formation of phenoxazinone rings more effectively. In contrast, heterodinuclear Cu(II)-Mn(II) complexes 4-6 exhibited significantly lower rate constants. The lowest activity was observed in complex 4 (0.0521 min^{-1}), which, containing *p*-chloroaniline, showed the weakest activity due to the effects of both the metal center and the substituent group.

The electronic properties of the substituents directly affected catalytic activity; *p*-toluidine, with an electron-donating methyl substituent, yielded the highest rate constants in complexes 2 and 5, thereby

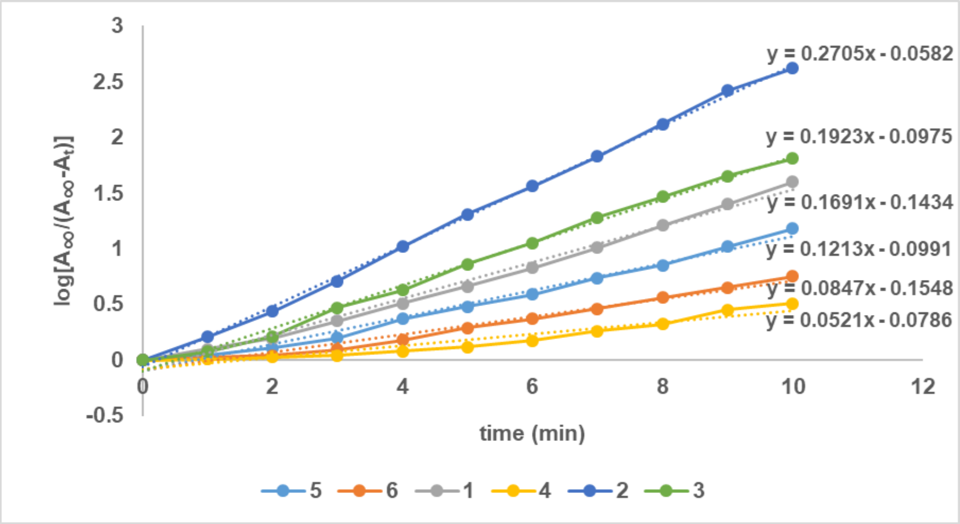


Figure 7. Comparison of observed rate constants for the phenoxazinone-like activity of the complexes

Table 3. Observed rate constants (k_{obs}) for the oxidation of OAPH to APX catalyzed by metal complexes

Compounds	Rate constant, k_{obs} (min^{-1})
1	0.1691
2	0.2705
3	0.1923
4	0.0521
5	0.1213
6	0.0847

facilitating redox cycling at the metal center. While *p*-acetylaniline, containing an electron-withdrawing carbonyl group, negatively affected the activity, the Cu(II)-Cu(II) structure in complex 3 partially compensated for this effect and provided a moderate rate constant. *p*-Chloroaniline, containing an electron-withdrawing and sterically effective chlorine group, caused the lowest catalytic activities in complexes 1 and 4.

It was determined that homodinuclear Cu(II)-Cu(II) complexes exhibited superior performance compared to heterodinuclear Cu(II)-Mn(II) complexes in phenoxazinone synthase-like activity. It was observed that electron-donating groups (especially *p*-toluidine) increased activity, whereas electron-withdrawing groups (*p*-chloroaniline and *p*-acetylaniline) reduced it. These findings showed that the functional groups on the ligand and the type of metal center directly affected the enzyme-like catalytic activity.

4. CONCLUSION

This work showed that dinuclear metal complexes based on diimine and dioxime groups have great potential as functional enzyme mimics. As demonstrated by thorough kinetic and spectrophotometric analyses, the types of metal centers and the electronic properties of ligand substituents significantly affect catalytic performance. The enhanced redox flexibility of Cu(II) centers in such biomimetic systems is evidenced by the consistently superior performance of homodinuclear Cu(II)-Cu(II) complexes relative to their heterodinuclear Cu(II)-Mn(II) counterparts across all examined enzymatic activities. Furthermore, the results indicated that even minor modifications in substituents, such as methyl, which donates electrons, rather than acetyl or chloro moieties, which withdraw electrons, could result in noticeable differences in catalytic behavior. The kinetic rate constants obtained for the three different enzymatic activities were also highly comparable to those reported for similar compounds in the literature, further supporting the effectiveness of the designed complexes (16-19). The tunability of these systems was demonstrated by the improved performance of complexes with electron-donating groups in redox-based transformations and with electron-withdrawing groups in peroxide-based processes. These findings enhance our understanding of the relationships between structure and activity in artificial enzyme models and provide practical methods for designing future catalysts. Catalytic profiles can be adapted for particular applications by logically adjusting metal-ligand structures. Such complexes offer promising opportunities in green catalysis, biosensing, and medicinal chemistry, bridging the gap between coordination chemistry and biological activity. The stability and reusability of these promising biomimetic catalysts may be further improved through future research on immobilization techniques, broader substrate scopes, and mechanistic insights gained from computational investigations.

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