Regular Article

PHYSICAL CHEMISTRY RESEARCH
Published by the
Iranian Chemical Society
www.physchemres.org
info@physchemres.org

Phys. Chem. Res., Vol. 12, No. 4, 859-868, December 2024
DOI: 10.22036/pcr.2024.420037.2433

Exploring Phenoxazinone Synthase Activities: Experimental and Theoretical Analyses of Symmetrical Azine Ligands

H. Meziane[a], Z. Abbaoui[a], M. Ouabane[b], A. Djedouani[c,d], S. Chetioui[e,f], M. El Kodadi[a,g], R. Touzani[a], B. Hammouti[h] and M. Bouachrine[b,*]

[a]*Laboratory of Applied Chemistry and Environment (LCAE), Department of Chemistry, Faculty of Sciences, University Mohammed Premier, Oujda, Morocco*

[b]*Molecular Chemistry and Natural Substances Laboratory (MCNSL), Department of Chemistry, Faculty of Sciences, Moulay Ismail University, BP 11201 Zitoune, Meknes-50000, Morocco.*

[c]*Laboratoire de Physicochimie Analytique et Cristallochimie des Matériaux Organométalliques et Biomoléculaires, Université Constantine 1, 25000, Algérie.*

[d]*Ecole Normale Supérieure de Constantine, Université Constantine 3, 25000, Algérie.*

[e]*Unité de Recherche de Chimie de l'Environnement et Moléculaire Structurale, (CHEMS), Faculté des Sciences Exactes, Département de Chimie, Université des Frères Mentouri, Constantine 1, Constantine 25000, Algérie.*

f Faculté de Technologie, Université Mohamed Boudiaf M'sila, Algérie

[g]*Laboratory of Innovation in Science, Technology and Education (LISTE), CRMEF Oriental, Oujda, Morocco.*

[h]*Euro-Mediterranean University of Fez (UEMF), BP. 15, 300700 Fez, Morocco*

(Received 11 October 2023, Accepted 7 May 2024)

This study investigates the catalytic potential of five recently synthesized symmetrical azine ligands in combination with various metal salts in situ for the synthesis of phenoxazinone synthase. UV spectrometry was used to analyze the resulting complexes, all of which exhibited remarkable efficiency in catalyzing the oxidation of aminophenol to phenoxazinone under ambient conditions. In particular, the pairing of the ligand L1 and $CuCl_2$ in a 1:1 molar ratio ([1L/1M]) showed the highest catalytic performance. These results offer promising prospects for the application of these complexes as effective catalysts in various chemical processes. Furthermore, a Density Functional Theory investigation of the chemical reactivity of these ligands was carried out to determine various widely recognized quantum chemical descriptors, including hardness, chemical potential, electrophilicity indices, and molecular orbital theory analysis. The study aimed to characterize nucleophilic, electrophilic, electron-accepting, and electron-donating compounds.

Keywords: Azine, Phenoxazinone synthase, Aminophenol, complexes *in situ*, DFT theoretical calculations

INTRODUCTION

Phenoxazinone synthase, an essential enzyme in organic chemistry, has garnered considerable attention for its pivotal role in catalyzing the conversion of aminophenol to phenoxazinone [1,2]. Phenoxazinones represent a diverse class of organic compounds that find wide-ranging applications in pharmaceuticals [3], agrochemicals [4], and dye synthesis [5,6]. The significance of phenoxazinone synthase lies in its ability to drive the formation of these valuable compounds with high efficiency and selectivity [7,8].

The relentless pursuit of sustainable and environmentally benign synthetic methodologies has led researchers to explore catalytic processes for phenoxazinone synthesis [9]. By harnessing the power of catalysts, scientists aim to

*Corresponding author. E-mail: m.bouachrine@umi.ac.ma

improve reaction rates, reduce energy consumption, and minimize waste generation [10]. Among the catalysts under investigation, symmetrical azine ligands have emerged as promising candidates due to their tunable electronic and steric properties [11,12].

The choice to combine these ligands with aminophenol is strategic and well-founded. Aminophenol represents a key substrate due to its prevalence in various chemical processes [13,14], and its direct involvement in phenoxazinone formation. By coordinating the symmetrical azine ligands with metal salts and combining them with aminophenol, the resulting complexes are anticipated to exhibit enhanced catalytic activity, potentially outperforming traditional methods [15].

In summary, this article underscores the importance of phenoxazinone synthase in organic synthesis and its relevance in producing valuable compounds for various industries (Fig. 1). The main objective is to explore the catalytic potential of five new symmetrical azine ligand-metal complexes in oxidizing aminophenol to phenoxazinone, employing green and sustainable methodologies. The insights acquired through this investigation will facilitate the systematic design of effective catalysts, fostering advancements in catalysis for synthesizing valuable organic compounds. Furthermore, a Density Functional Theory analysis of the chemical reactivity of these ligands was conducted to determine various well-established quantum chemical descriptors, including hardness, chemical potential, electrophilicity indices, and molecular orbital theory analysis. The DFT calculations were made, interpreted, and aimed to identify nucleophilic, electrophilic, electron-accepting, and electron-donating compounds [15].

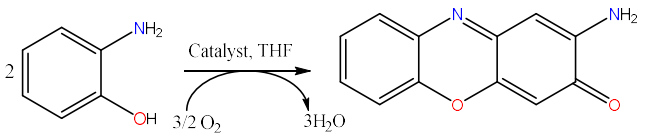


Fig. 1. General reaction of phenoxazinone synthase.

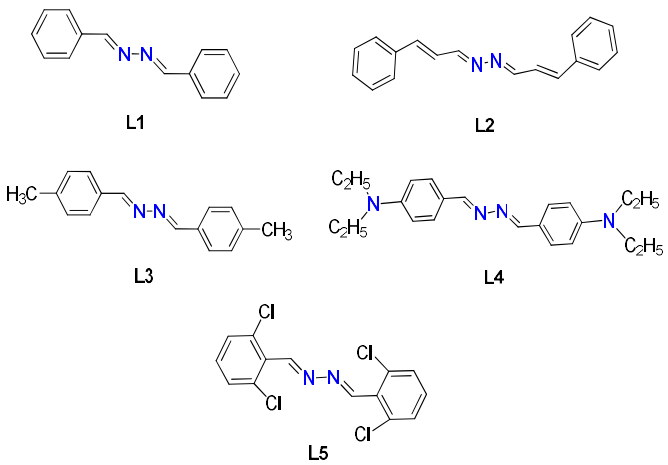


Fig. 2. Structures of different ligands used in the study.

MATERIALS AND METHODS

Materials

Salts such as $CuSO_4$, $CuCl_2$, $Cu(NO_3)_2$, $MnCl_2$, $CoCl_2$, $MnCl_2$, $Ni(ClO_4)$, $Cu(OAc)_2$, and o-aminophenol(OAP), were received from Aldrich and used without further purifications.

All the ligands used in this study were synthesized according to the literature procedure [16]. Aldehyde was dissolved in Ethanol (10 ml) and added dropwise to a solution of hydrazine monohydrate (1 mmol) with a catalytic amount of glacial acetic acid in ethanol (10 ml) at room temperature. The reaction mixture was refluxed with stirring for 4 h and monitored by TLC. The precipitate thus formed was filtered, washed with ethanol, and finally was recrystallized from absolute ethanol [17]. The structures of the ligands are shown in Fig. 2.

Activity measurements of phenoxazinone were conducted using a Shimatzu 1900i Spectrophotometer through kinetic measurements [18]. The spectrophotometric analysis was performed at 25 °C, monitoring the appearance of phenoxazinone over time at 430 nm (maximum absorbance, ε = 1900 M^{-1} cm^{-1} in THF).

Methods

The complexes were prepared in situ by sequentially mixing 0.15 ml of a 4 × 10^{-3} M metal salts solution with 0.15 ml of a 4 × 10^{-3} M ligand solution. Subsequently, the complexes formed in situ were treated with 2 ml of a 10^{-3} M aminophenol solution in tetrahydrofuran (THF) under aerobic conditions. This methodology was detailed according to our previous works [19,20].

Global Indices of the Studied Molecules Using the DFT Approach

In this study, Density Functional Theory (DFT) was employed to compute global reactivity indices for various compounds [21,22]. These indices encompass chemical potential (Eq. (1)), softness (Eq. (2)), chemical hardness (Eq. (3)), global electrophilicity (Eq. (4)), and hyper-hardness (Eq. (5)) [23]. The study aimed to identify covalent energy (Eq. (6)), electrofugality (Eq. (7)), Maximum load capacity (Eq. (8)), and Nucleofugality (Eq. (9)) of compounds [24]. Calculations were based on minimum-energy geometries using the B3LYB6-31G(d,p) optimization method, [25] and the results provide insights into the reactivity and electronic stability of the studied molecules [26,27].

$$\mu = (E_{HOMO} + E_{LUMO})/2 \quad (1)$$

$$S = 1/\eta \quad (2)$$

$$\eta = E_{LUMO} - E_{HOMO} \quad (3)$$

$$\omega = \mu^2/2\eta \quad (4)$$

$$\gamma = \varepsilon_{LUMO} - 2\varepsilon_{HOMO} + \varepsilon_{HOMO-1} \quad (5)$$

$$\Delta E^{A}_{Covalent} \approx -\frac{\chi^2}{\eta} \quad (6)$$

$$\Delta E = \mu\Delta N + \frac{1}{2}\eta\Delta N^2 \quad (7)$$

$$\Delta N_{opt} = -\frac{\mu}{\eta} \quad (8)$$

$$N = \frac{1}{\omega} \quad (9)$$

RESULTS AND DISCUSSION

Study of Phenoxazinone Synthase in the Presence of *In-Situ* Formed Complexes with Ligands L1-L5 and Metallic Salts in THF Solvent

Catalytic study of combinations (1L/1M) in THF. The phenoxazinone synthase activity of the complexes, formed in situ with L1-L5 ligands and metallic salts, was investigated through spectrophotometric analysis. The appearance of the maximum absorbance at 430 nm for the corresponding phenoxazinone was monitored over time. Figures (3-8) show the changes in absorbance at 430 nm over a period of 60 min. Notably, the spectrum for aminophenol alone displayed minimal absorbance variation with time. The absorbance changes over time for the oxidation of aminophenol in the presence of the complexes formed by ligands L1-L5 with metallic salts (II) revealed that the combination of ligand L1 with $CuCl_2$ showed more significant activity compared to other combinations.

Table 1 displays the reaction rate V (μmol l^{-1} min^{-1}) for the oxidation of aminophenol in THF (1Ligand/1Salt). The results in Table 1 indicate that all copper complexes formed in situ with L1-L5 ligands catalyzed the oxidation of aminophenol to phenoxazinone. Among them, the complex formed from L1 and the metal salt $CuCl_2$ demonstrated the highest phenoxazinone activity.

UV-VIS Spectrophotometric Study

The catalytic activity of the combination (1L/1M) involving ligand L1 and $CuCl_2$ and the combination (1M/1L) in THF was validated. Kinetic experiments were conducted at room temperature, and the formation of phenoxazinone and the corresponding absorbance changes were recorded

Table 1. The Reaction Rate for the Oxidation of Aminophenol in THF

L/M	$Ni(ClO_4)_2$	$MnCl_2$	$Cu(NO_3)_2$	$CuCl_2$	$Cu(CH_3COO)_2$
L1	1.79	0.72	17.60	28.10	6.61
L2	2.35	1.35	16.80	26.10	6.60
L3	2.02	1.25	18.70	27.00	8.82
L4	1.60	1.26	14.20	24.30	9.20
L5	1.34	1.24	20.30	24.50	8.36

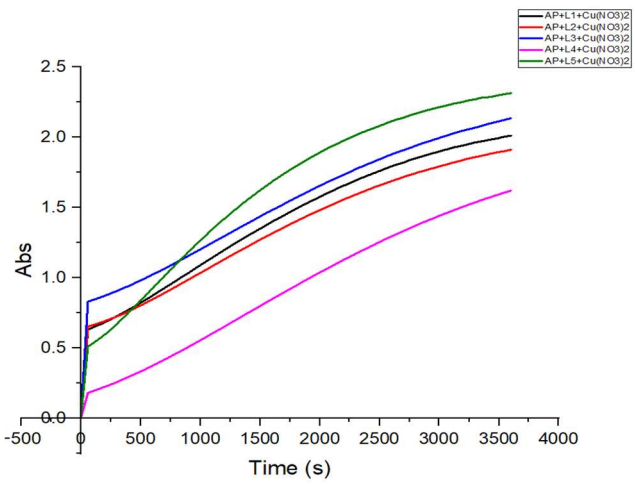


Fig. 3. Aminophenol oxidation of mixing $Cu(NO_3)_2$ with different ligands (1L/1M) in THF.

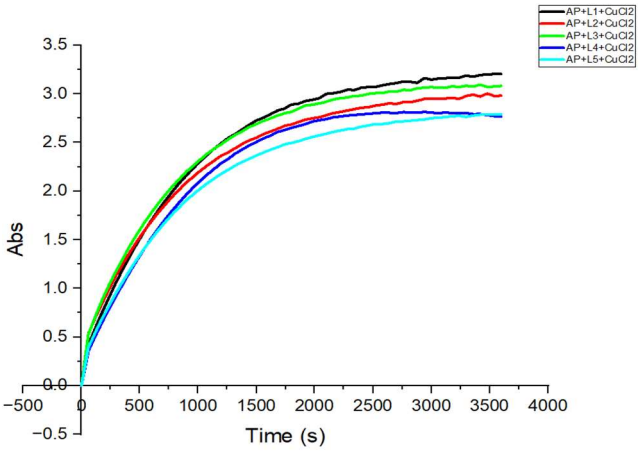


Fig. 4. Aminophenol oxidation by mixing $CuCl_2$ with different ligands (1L/1M) in THF.

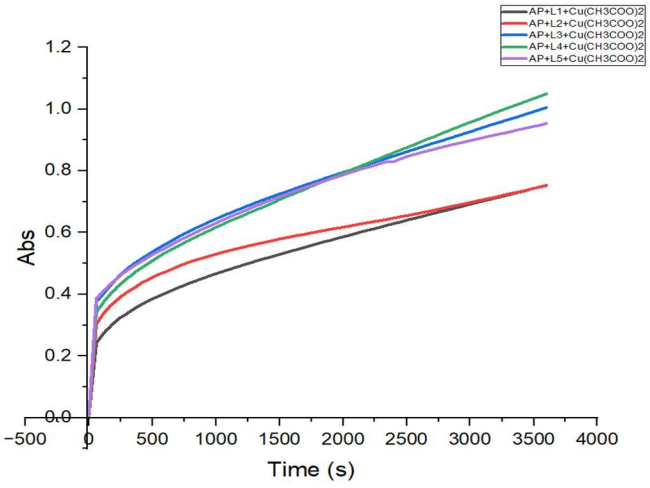


Fig. 5. Aminophenol oxidation of mixing $Cu(OAc)_2$ with different ligands (1L/1M) in THF.

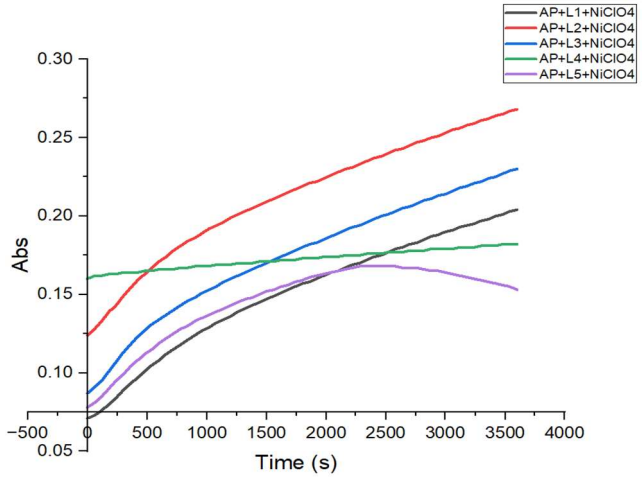


Fig. 6. Aminophenol oxidation of mixing $Ni(ClO_4)_2$ with different ligands (1L/1M) in THF.

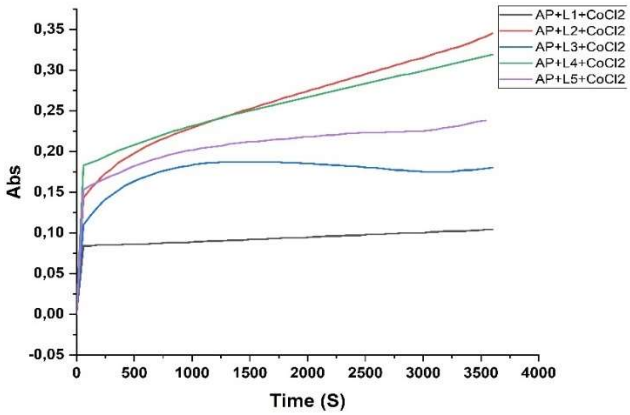


Fig. 7. Aminophenol oxidation of mixing $CoCl_2$with different ligands (1L/1M) in THF.

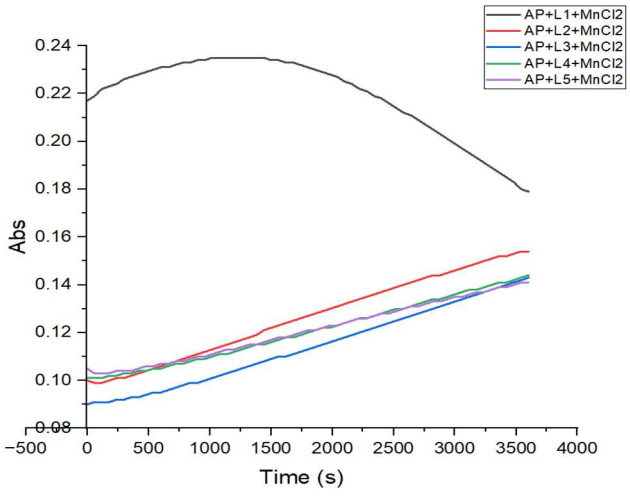


Fig. 8. Aminophenol oxidation of mixing $MnCl_2$with different ligands (1L/1M) in THF.

every 5 min (Fig. 9). The appearance of a strong band at 430 nm (Fig. 3) indicates that the combination (1L/1M) of ($L1/CuCl_2$) serves as an efficient catalyst for the oxidation of aminophenol to phenoxazinone.

Kinetic Study

A kinetic study was carried out by determining the constants Km and Vmax of the oxidation reaction of aminophenol. The method consists of plotting the graph representing the Vi as a function of the substrate concentration (aminophenol). We carried out this study by using a solution of the combinations $L1/CuCl_2$, $L2/CuCl_2$, $L3/CuCl_2$, $L4/CuCl_2$, $L5/CuCl_2$, $L1/Cu(NO_3)_2$, $L3/Cu(NO_3)_2$ and $L5/Cu(NO_3)_2$, treated with different concentrations aminophenol (from 0.25×10^{-3} M to 6×10^{-3} M) under ambient conditions. For the graphical determination of these two constants (K_m and V_{max}), there are Michaelis-Menten graphical representations, Figs. 10 and 11, that present the results, allowing more precise extrapolations.

By measuring the initial speed (V_0) of the reaction for different concentrations of the substrates, we constructed a graph of V_0 (µM min^{-1}) as a function of the variation in the concentration of the substrates (M), which allowed us to obtain the constants K_m and V_{max} for the different complexes formed in situ (Table 2).

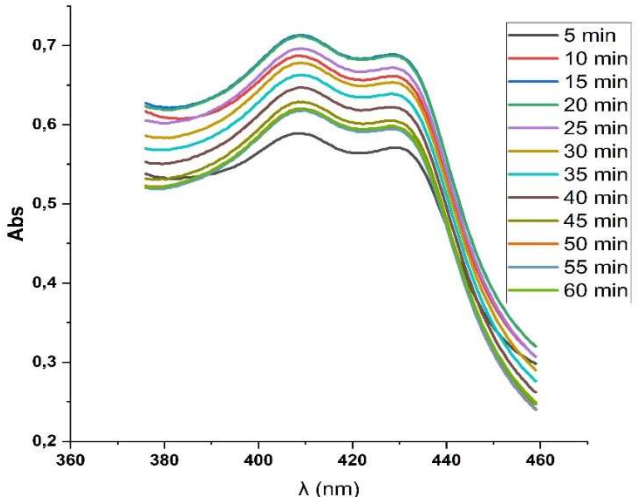


Fig. 9. Absorbance spectrum of phenoxazinone as a function of time for the combination $L1/CuCl_2$ in THF.

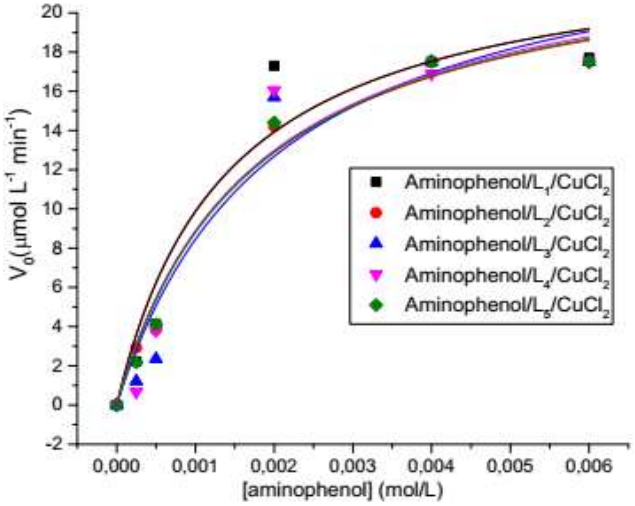


Fig. 10. Dependence of the reaction rates on the aminophenol (2 ml) concentrations varying 0.25×10^{-3} M to 6×10^{-3} M for the oxidation reaction catalysed by $L_i/CuCl_2$ (i = 1-5) complexes (0.3 ml; 4×10^{-3} M) at 430 nm in THF.

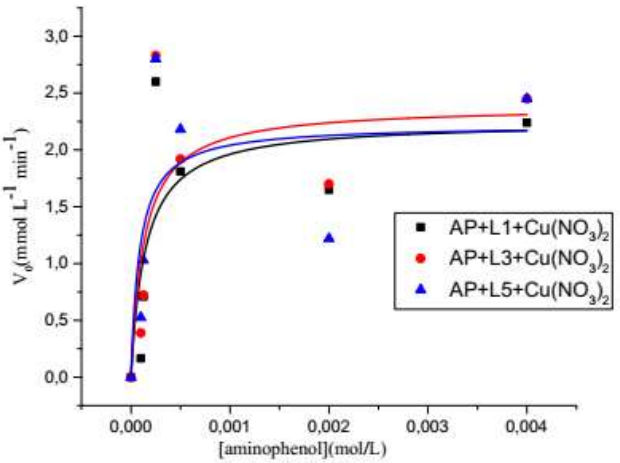


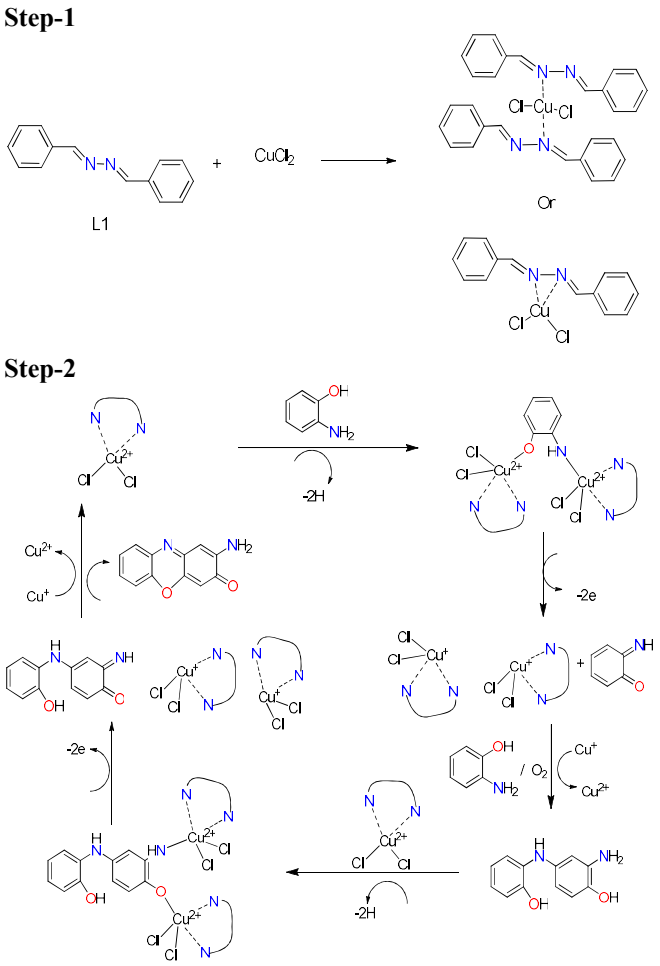
Fig. 11. Dependence of the reaction rates on the aminophenol (2 ml) concentrations varying 0.25×10^{-3} M to 4×10^{-3} M for the oxidation reaction catalysed by $L_i/Cu(NO_3)_2$ (i = 1, 3 and 5) complexes (0.3 ml; 4×10^{-3} M) at 430 nm in THF.

Proposed Reaction Pathway

To understand the intricacies of the reaction, we studied previously reported spectroscopy and theoretical studies [28,29]. We proposed a mechanism for the oxidation of aminophenol to phenoxazinone (Scheme 1). The mechanism is unscrewed in two steps, the first (step-1) involves the formation of the complex, and the second step (step-2) involves the binding of aminophenol to the complex.

Table 2. Maximum Reaction Rates (V_{max}) and K_m Constants

	V_{max} ($\mu M\ min^{-1}$)	K_m (M)
Aminophenol/L_1/$CuCl_2$	19.54	0.00099
Aminophenol/L_2/$CuCl_2$	18.79	0.0011
Aminophenol/L_3/$CuCl_2$	19.22	0.0012
Aminophenol/L_4/$CuCl_2$	18.96	0.0011
Aminophenol/L_5/$CuCl_2$	19.00	0.0011
Aminophenol/L_1/$Cu(NO_3)_2$	2.18	0.00014
Aminophenol/L_3/$Cu(NO_3)_2$	2.33	0.00013
Aminophenol/L_5/$Cu(NO_3)_2$	2.22	0.00008



Scheme 1. Proposed mechanism pathway of the catalytic oxidation of phenoxazinone synthase by complexes formed *in situ*.

Quantum Chemical Calculations

Conceptual Density Functional Theory (DFT) offers theoretical chemists the convenience of predicting chemical reactivity in chemical systems through straightforward and practical equations. The electronic properties of the molecules under examination, such as the ionization potential (I), are quantified as the energy needed to remove an electron from a molecule. Furthermore, a high ionization energy indicates high stability and therefore chemical inertness, while a low ionization energy indicates a molecule's propensity to be reactive. Electron affinity (A) is defined as the energy released when an electron is added to a neutral molecule, so a high value of (A) indicates the tendency of the molecule to retain its electrons. A negative chemical potential (μ) indicates molecular stability or the resistance of the molecule to being broken down into its constituent parts. Hardness (η) characterizes the resistance of the molecular electron cloud to deformation during small perturbations. A large HOMO-LUMO energy gap indicates a hard molecule with low polarizability, low chemical, and biological activities, but high kinetic sensitivity, while a small HOMO-LUMO energy gap indicates a soft molecule with high polarizability, high chemical and biological activities, but low kinetic sensitivity. The global electrophilicity index (ω) of a molecule is a measure of its energy stabilization upon the addition of an external electron charge or its resistance to electron exchange with copper. Most of these properties are influenced by the presence or type of functional groups, the size of the molecule, and the presence of pi bonds (delocalization). Optimal structure, total electron density, most occupied molecular orbital (HOMO), and least occupied molecular orbital (LUMO) are some of the crucial features used to determine the quantum parameters of the molecules present in Table 2. In this study, the following messages are displayed for these parameters: The LUMO orbital represents where molecules can receive electrons from the copper d orbital. The dense HOMO region indicates the region of the molecule where the ability to donate electrons is high.

Table 2. Quantum Descriptors Obtained with DFT/B3LYB6-31G(d,p)

No	E (ev)	DM (C.m)	E_{LUMO}	E_{HOMO}	E_{HOMO-1}	μ	η	S	ω
L1	-17692.656	6.10E+30	-1.781	-5.968	-6.206	-3.875	4.187	0.239	1.793
L2	-21905.603	6.67E+26	-2.187	-5.464	-6.167	-3.825	3.277	0.305	2.233
L3	-19832.618	8.05E+30	-1.672	-5.775	-6.018	-3.723	4.103	0.244	1.689
L4	-28019.171	1.18E+31	-1.624	-5.379	-5.920	-3.502	3.755	0.266	1.633
L5	-67716.565	1.07E+31	-2.048	-6.447	-6.725	-4.247	4.400	0.227	2.050

No	γ	ΔE^-	ΔE^+	ω^+	ω^-	S^2	Δω	ΔN	P	IRI
L1	3.949	0.012	7.761	1.910	5.785	-0.054	7.695	0.925	0.253	-1.851
L2	2.575	0.046	7.697	2.757	6.583	-0.073	9.340	1.167	0.388	-2.334
L3	3.860	0.018	7.464	1.774	5.497	-0.056	7.271	0.907	0.259	-1.815
L4	3.214	0.009	7.012	1.749	5.251	-0.061	7.000	0.932	0.311	-1.865
L5	4.122	0.003	8.497	2.252	6.499	-0.048	8.751	0.965	0.243	-1.931

Molecular Orbitals

The description of molecular orbitals in the literature provides evidence of the chemical reactivity of molecules. This is referred to as local reactivity or selectivity, which provides information and projects a molecule's reactivity. Although John Dalton's prediction that an atom would be a vacuum is clearly refuted by the electronic dispersion of a molecule. This orbital distribution is one of the elements that regulate the chemical behavior of molecules. Consequently, a molecule that has reached its final octet state is no longer practical for a given reaction. For molecules L1, L2, ..., and L5, the optimized structure, HOMO and LUMO orbitals, as well as the total electron density, are presented in Fig. 12. The coverage of the electron density by the entire molecule indicates the potential of the molecule to enhance adsorption capacity on metal surfaces (copper). The regions of the HOMO and LUMO orbitals also show a clear indication that the adsorption capacity of the oleuropein molecule may depend on the behavior of the HOMO and LUMO orbitals of the molecules. The HOMO and LUMO orbitals also occupy hetero atoms present in the molecules, either in nitro groups or in benzene rings.

The analysis of the energetic and reactivity properties of the five molecules in question reveals significant differences. Firstly, in terms of energy stability, L5 stands out as the most stable, closely followed by L4, while L2 is the least stable of all. This difference in stability could have a considerable impact on the reactivity of these molecules in various contexts.

Regarding polarity, as measured by the dipole moment (DM), L4, and L5 display high values, suggesting significant polarity. L1 exhibits the lowest dipole moment, closely followed by L3. This variation in polarity could influence molecular interactions, especially in environments where electrostatic interactions are crucial.

Concerning chemical reactivity, the values of the frontier molecular orbitals (ELUMO and EHOMO) play a crucial role. In general, ELUMO values are more negative than EHOMO values for all molecules, indicating a tendency to accept electrons, classifying them as potential electrophiles. However, L5 stands out with the lowest ELUMO value (-2.048 eV), indicating an exceptionally high affinity for electrons. Thus, L5 emerges as the strongest electrophile among all the studied molecules.

Molecules L1, L2, L3, and L4, with relatively close ELUMO and EHOMO values, display similar electrophilic and nucleophilic reactivities.

These results provide valuable insights into the reactivity of these molecules, crucial for understanding their potential interactions in specific chemical reactions or biological processes. They could also serve as a basis for the design of molecules in various applications, especially in fields where reactivity and stability are critical factors.

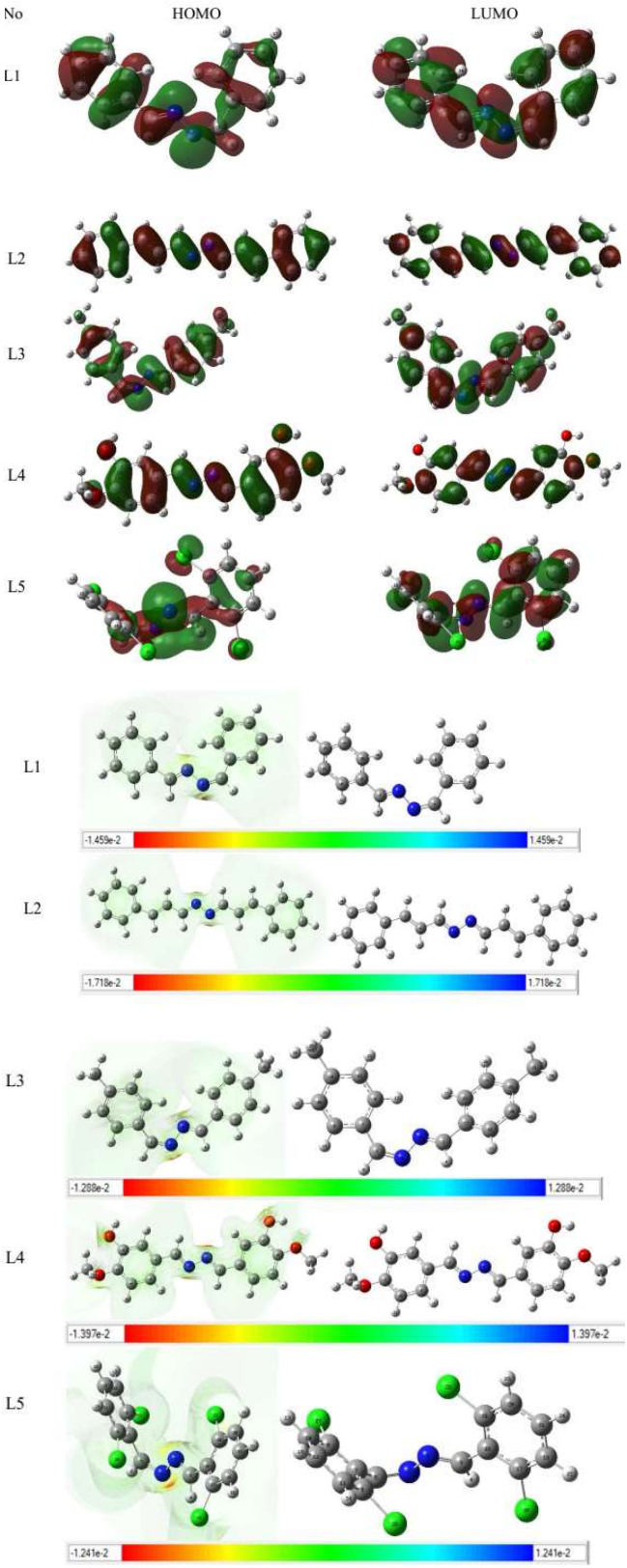


Fig. 12. Optimized, ESP, and HOMO/LUMO orbitals.

CONCLUSION

The present paper investigates the catalytic activity of azine ligands with different copper(II) salts. We found that under ambient conditions, using atmospheric oxygen as an oxidant (synthase activity), all combinations can catalyze the aminophenol oxidation reaction to phenoxazinone, but at different rates. Our study indicates that the nature of the solvent plays a crucial role in the catalytic activity of the complexes studied *in situ* and that the nature of the ligand significantly influences the catalytic efficiency of the corresponding combinations. On the other hand, combinations in which the ligand is associated with copper(II) chloride are observed to be more effective in catalyzing the oxidation of catechol in the presence of molecular oxygen, demonstrating that the nature of the counterion also influences the synthase activity. Furthermore, the ligand/metal ratios (2eq/1eq) were found to be excellent catalysts for the oxidation of aminophenol to phenoxazinone, and our study shows that the catalytic activity of the different combinations studied is influenced by the ligand concentration. We have also investigated the kinetics using the Michaelis-Menten model and our results are consistent with the Michaelis-Menten model. From the calculated DFT-based chemical reactivity descriptors, the efficacy is attributed to their low dipole moment value, significant pi-sigma-pi conjugation, high chemical affinity, and strong electron-donating ability. The calculated density functional parameters will be useful for the design and synthesis of such molecules in future experimental studies.

REFERENCES

[1] Dey, S. K.; Mukherjee, A., Catechol Oxidase and Phenoxazinone Synthase: Biomimetic Functional Models and Mechanistic Studies. *Coordination Chemistry Reviews*. *M* **2016**, *310*, 80-115, DOI: 10.1016/ j.ccr.2015.11.002.

[2] Kumbhakar, S.; Giri, B.; Muley, A.; Karumban, K. S.; Maji, S., Design, Synthesis, Structural, Spectral, and Redox Properties and Phenoxazinone Synthase Activity of Tripodal Pentacoordinate Mn(II) Complexes with Impressive Turnover Numbers. *Dalton Transactions. O* **2021**, *50* (45), 16601-16612,

https://doi.org/10.1039/D1DT01925B.

[3] Polak, J.; Jarosz-wilkołazka, A., Synthesis of Phenoxazinone-Type Pharmaceutical Compounds by Fungal Biomass. *Annales Universitatis Mariae Curie-Sklodoska Lubmin-Polonia*. *J* **2010**, *XXIII* (2), 2-7, DOI://https://doi.org/10.1186/1475-2859-9-51.

[4] Khairy, M.; Mahmoud, A. H.; Khalil, K. M. S., Synthesis of Highly Crystalline La FeO3 Nanospheres for Phenoxazinone Synthase Mimicking Activity. *RSC Advances*. *M* **2021**, *11* (29), 17746-17754, DOI: 10.1039/d1ra02295d.

[5] Jia, H.; Gao, Z.; Ma, Y.; Zhong, C.; Xie, Y.; Zhou, H.; Wel, P., Optimization of Phenoxazinone Synthase Production by Response Surface Methodology and Its Application in Congo Red Decolourization. *Electronic Journal of Biotechnology*. *S* **2013**, *16* (5), 2-2, DOI: 10.2225/vol16-issue5-fulltext-11.

[6] Fernandes, A., Pinto, B., Bonardo, Royo, L., Robalo, M. P.; Martins, L. O., Wasteful Azo Dyes as a Source of Biologically Active Building Blocks. *Frontiers in Bioengineering and Biotechnology*. *J.* **2021**, *9*, 1-9, DOI: 10.3389/fbioe.2021.672436.

[7] Witayakran, S.; Ragauskas, A. J., Synthetic Applications of Laccase in Green Chemistry. *Advanced Synthesis and Catalysis*. *J.* **2009**, *351* (9), 1187-1209, DOI: 10.1002/ adsc.200800775.

[8] Maurya, M. R.; Chauhan, A.; Verma, A.; Kumar, U.; Avecilla, F., Amine-Functionalized Titanium Dioxide Supported Dioxidomolybdenum(VI) Complexes as Functional Model for Phenoxazinone Synthase Enzyme. *Catalysis Today*. *A* **2022**, *388-389*, 274-287, DOI: 10.1016/j.cattod.2020.06.031.

[9] AlAli, A.; Khamees, H. A.; Madegowda, M.; Zarrouk, A.; Kumara, K.; El-khatatneh, N.; Warad, I.; Khanum, S. A., One-pot reproducible Sonosynthesis of trans-[Br(N∩N')Cu(μBr)2Cu(N∩N')Br] dimer:[H….Br S(9)] synthons, spectral, DFT/XRD/HSA, thermal, docking and novel LOX/COX enzyme inhibition. Journal of Molecular Structure. *N* **2022**, *1275,* 134626, DOI: 10.1016/j.molstruc.2022.134626.

[10] Bassanini, I.; Ferrandi, E. E.; Riva, S.; Monti, D., Biocatalysis with Laccases: An Updated Overview. *Catalysts*. *D* **2020**, *11* (1), 1-30, DOI: 10.3390/ catal11010026.

[11] Lawrence, M. A. W.; Green, K. A.; Nelson, P. N.; Lorraine, S. C., Review: Pincer Ligands-Tunable, Versatile and Applicable. *Polyhedron*. *M* **2018**, *143*, 11-27, DOI: 10.1016/j.poly.2017.08.017.

[12] Chouiter, A. D.; Mousser, M. O.; Mousser, H. B.; Krid, A.; Belkhir, L.; Fleutot, S.; François, M., Synthesis, Spectra, Crystal, DFT, Molecular Docking and in Vitro Cholinesterase Inhibition Evaluation on two Novel Symmetrical Azine Schiff bases. *Journal of Molecular Structure. J.* **2023**, *1281*, 135171, DOI: 10.1016/j.molstruc.2023.135171.

[13] Sun, J.; Zhao, J.; Bao, X.; Wang, Q.; Yang, X., Alkaline Phosphatase Assay Based on the Chromogenic Interaction of Diethanolamine with 4-Aminophenol. *Analytical Chemistry*. *A* **2018**, *90* (10), 6339-6345, DOI: 10.1021/acs.analchem.8b01371.

[14] Ipinloju, N.; Balogun, T. A.; Ibrahim, A.; Adeyemo, M. A.; Esan, T. O.; Emmanuel, A. V.; Bello, K. A.; Omiyeniyi, G. D.; Oyeneyin, O. E., Computational Investigation of Polo-like Kinase 1 (plk1): Inhibitive Potential of Benzimidazole-Carbonamide Derivatives for Cancer Treatment. *Physical Chemistry Research. J.* **2024**, *12* (2), 333-348, DOI: 10.22036/pcr.2023.377616.2331.

[15] Azam, M.; Hussain, Z.; Warad, I.; Al-Resayes Saud, I.; Shahnawaz, Kh. M.; Shakir, M.; Trzesowska-Kruszynska, A., Kruszynski, R., Novel Pd(II)-salen complexes showing high in vitro anti-proliferative effects against human hepatoma cancer by modulating specific regulatory genes. *Dalton Transactions*. *J.* **2012**, *41* (35), 10854-10864, DOI: 10.1039/c2dt31143g.

[16] Chourasiya, S. S.; Kathuria, D.; Wani, A. A.; Bharatam, P. V., Azines: Synthesis, Structure, Electronic Structure and Their Applications. *Organic and Biomolecular Chemistry. J.* **2019**, *17* (37), 8486-8521, DOI: 10.1039/c9ob01272a.

[17] El Faydy, M.; Benhiba, F.; About, H.; Kerroum, Y.; Guenbour, A.; Lakhrissi, B.; Warad, I.; Verma, Ch.; El-Sayed, M. Sh.; Ebenso, Eno E.; Zarrouk, A., Experimental and computational investigations on the anti-corrosive and adsorption behavior of 7-N,N'-dialkyaminomethyl-8- Hydroxyquinolines on C40E steel surface in acidic medium. *Journal of Colloid and Interface Science. M* **2020**, *576* (2020), 330-344, DOI: 10.1016/j.jcis.2020.05.010.

[18] AlAli, A.; Al-Noaimi, M.; AlObaid, A.; Khamees, H. A.; Zarrouk, A.; Kumara, K.; Warad, I.; Khanum, S. A., Jahn-Teller distortion in SP-like [Cu(bipy)(triamine)].2BF4 complexes with novel N-H…F/C-H…F synthon: XRD/HSA-interactions, physicochemical, electrochemical, DFT, docking and COX/LOX inhibition. *Journal of Molecular Liquids*. *J.* **2023**, *387* (2023), 122689, DOI: 10.1016/ j.molliq.2023.122689.

[19] Abu Saleemh, F.; Musameh, Sh.; Sawafta, A.; Brandao, P.; Tavares, C. J.; Ferdov, S.; Barakat, A.; Al Ali, A.; Al-Noaimi, M.; Warad, I., Diethylenetriamine/ diamines/copper(II) complexes [Cu(dien)(NN)]Br_2: Synthesis, solvatochromism, thermal, electrochemistry, single crystal, Hirshfeld surface analysis and antibacterial activity. *Arabian Journal of Chemistry. O* **2016**, *10* (6), 845-854, DOI: 10.1016/j.arabjc.2016.10.008.

[20] Bouroumane, N.; El Boutaybi, M.; El Kodadi, M.; Touzani, R.; Oussaid, A.; Hammouti, B.; Abboud, M., Synthesis of New Heterocyclic Ligands and Study of the Catecholase Activity of Catalysts Based on Copper(II). *Reaction Kinetics, Mechanisms and Catalysis. A* **2023**, *136* (3), 1545-1562, DOI: 10.1007/s11144-023-02370-7.

[21] Becke, A. D., A New Mixing of Hartree-Fock and Local Density-Functional Theories. *Journal of Chemical Physics*. *O* **1992**, *98* (2), 1372-1377, DOI: 10.1063/1.464304.

[22] Azaid, A.; Abram, T.; Kacimi, R.; Bejjit, L.; Bennanib, M. N.; Bouachrine, M., DFT/TDDFT Studies of the Structural, Electronic, NBO and non-Linear Optical Proper-ties of Triphenylamine Functionalized tetra-Thiafulvalene. *Turkish Computational and Theoretical Chemistry. A* **2018**, *2* (2), 36-48, https://doi.org/10.33435/tcandtc.926405.

[23] Raftani, M.; Abram, T.; Azaid, A.; Kacimi, R.; Bennani, M. N.; Bouachrine, M., Theoretical Design of New Organic Compounds Based on diKetopyrrolopyrrole and Phenyl for Organic Solar Bulk Heterojunction Cell Applications: DFT and TD-DFT study. *Materials Today: Proceedings. M* **2021**, *45*, 7334-7343, DOI: 10.1016/j.matpr.2020.12.1228.

[24] Pathade, S. S.; Jagdale, B. S., Experimental and Computational Investigations on the Molecular Structure, Vibrational Spectra, Electronic Properties, FMO and MEP Analyses of 4,6-Bis(4-Fluorophenyl)-5,6-diHydropyrimidin-2(1H)-one: A DFT Insight. *Physical Chemistry Research. D* **2020**, *8* (4), 671-687, DOI: 10.22036/pcr.2020.227546.1763.

[25] Khaldan, A.; Bouamrane, S.; El-Mernissi, R.; Alaqarbeh, M.; Hajji, H.; Alsakhen, N.; Maghat, H.; Ajana, M. A.; Sbai, A.; Bouachrine, M.; Lakhlifi, T., Computational Study of Quinoline-Based Thiadiazole Compounds as Potential Antileishmanial Inhibitors. *New Journal of Chemistry. J* **2022**, *46* (36), 17554-17576, DOI: 10.1039/d2nj03253h.

[26] Ouabane, M.; Alaqarbeh, M.; Hajji, H.; Tabti, K.; Ajana, M. A.; Sbai, A.; Sekkate, C.; Lakhlifi, T.; Bouachrine, M., Quality Control of Coumarins, Furocoumarins and Polymethoxyflavones in Citrus Essential Oils: In Silico Analysis. *Chemistryselect. F* **2024,** *9* (5), e202303037, https://doi.org/10.1002/slct.202303037.

[27] Lee, C.; Yang, W.; Parr, R. G., Development of The Colle-Salvetti Correlation-Energy Formula into a Functional of The Electron Density. *EE: Evaluation Engineering*. *J* **1988**, *50* (11), 36-39, DOI: 10.1103/ PhysRevB.37.785.

[28] Klabunde, T.; Eicken, C.; Sacchettini, J. C.; Krebs, B., Crystal Structure of a Plant Catechol Oxidase Containing a diCopper Center. *Nature Structural Biology. D* **1998**, *5* (12), 1084-1090, DOI: 10.1038/4193.

[29] Solomon, E. I.; Sundaram, U. M.; Machonkin, T. E., Multicopper Oxidases and Oxygenases. *Chemical reviews*. *A* **1996**, *96* (7), 2563-2606, DOI: 10.1021/cr950046o.