New Pyrazole-Hydrazone Derivatives: X-ray Analysis, Molecular Structure Investigation via Density Functional Theory (DFT) and Their High In-Situ Catecholase Activity

Khalid Karrouchi 1,2,3, El Bekkaye Yousfi 4, Nada Kheira Sebbar 5, Youssef Ramli 1, Jamal Taoufik 1, Younes Ouzidan 6, M’hammed Ansar 1, Yahia N. Mabkhot 7,*, Hazem A. Ghabbour 8, and Smaail Radi 2,*

1 Laboratoire de Chimie Thérapeutique, Faculté de Médecine et de Pharmacie, Université Mohammed V, P. O. Box 8007, Rabat 10100, Morocco; khalid.karrouchi@um5s.net.ma (K.K.); yramli76@yahoo.fr (Y.R.); jtaoufik@hotmail.com (J.T.); ansarmhammed@gmail.com (M.A.)
2 Laboratoire de Chimie Appliquée et Environnement (LCAE), Faculté des Sciences, Université Mohamed I, P. O. Box 524, Oujda 60000, Morocco
3 Laboratoire National de Contrôle des Médicaments, Direction du Médicament et de la Pharmacie, Ministère de la Santé, P. O. Box 6206, Rabat 10100, Morocco
4 Institution Supérieure des Professions Infirmières et Techniques de Santé, P. O. Box 4806, Oujda 60000, Morocco; yousfi@netcourrier.com
5 Laboratoire de Chimie Organique Hétérocyclique, Pharmacochimie, Faculté des sciences, Université Mohammed V, P. O. Box 8007, Rabat 10100, Morocco; snouncousebbar@gmail.com
6 Laboratoire de Chimie Organique Appliquée, Faculté des Sciences et Techniques, Université Sidi Mohamed Ben Abdellah, P. O. Box 2202, Fès 30000, Morocco; younes.ouzidan@usmba.ac.ma
7 Department of Chemistry, Faculty of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
8 Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh 11451, Saudi Arabia; ghabbourh@yahoo.com
*
Correspondence: yahia@ksu.edu.sa (Y.N.M.); s.radi@ump.ac.ma (S.R.); Tel.: +212-536-500-601 (S.R.)

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Abstract: The development of low-cost catalytic systems that mimic the activity of tyrosinase enzymes (Catechol oxidase) is of great promise for future biochemistry technologic demands. Herein, we report the synthesis of new biomolecules systems based on hydrazone derivatives containing a pyrazole moiety (L1–L6) with superior catecholase activity. Crystal structures of L1 and L2 biomolecules were determined by X-ray single crystal diffraction (XRD). Optimized geometrical parameters were calculated by density functional theory (DFT) at B3LYP/6–31G (d, p) level and were found to be in good agreement with single crystal XRD data. Copper (II) complexes of the compounds (L1–L6), generated in-situ, were investigated for their catalytic activities towards the oxidation reaction of catechol to ortho-quinone with the atmospheric dioxygen, in an attempt to model the activity of the copper containing enzyme tyrosinase. The studies showed that the activities depend on four parameters: the nature of the ligand, the nature of counter anion, the nature of solvent and the concentration of ligand. The Cu(II)-ligands, given here, present the highest catalytic activity (72.920 μmol·L⁻¹·min⁻¹) among the catalysts recently reported in the existing literature.

Keywords: pyrazole; hydrazone; crystal structure; DFT; catecholase activity
1. Introduction

Bioorganometallic compounds are reputed for their remarkable applications in the field of catalysis; however, much less is known about their potential in chemical biology [1,2]. An important goal in organometallic chemistry is the synthesis of biomolecules that exhibit catalytic activity analogous to the activity of enzymes. A number of catalysts having biomimetic activity for different enzymes have been designed by chemists [3,4]. The preparation and etude of efficient template complexes for metalloenzymes with oxidase or oxygenase activity are thus most important for the elaboration of new veritable catalysts for oxidation reactions [5]. Copper ions, as centers of potent site of various metalloproteins, play an essential role in several biological processes: electron transfer, oxidation, transport of dioxygen, etc. [6,7]. Copper complexes of low molecular weight are studied as structural and functional models of active centers of enzymes with copper [8–11].

Catechol oxidases perform the oxidation of 1,2-diphenols, such as catechol, to o-quinones, using dioxygen (O₂). Hydrogen atoms removed from catechol combine with oxygen to form water. The crystal structures of different forms of catechol oxidase have improved the accordance of the mechanism of catecholase activity of catechol oxidase. Several workers proposed the mechanism of catechol oxidation by natural enzyme, including important proposals by Solomon and kerbs [12,13]. In addition to the high number of Cu(II) complexes reported, their catecholase activity has also been demonstrated, although their mechanistic aspects are not as well understood as those of Cu(II) complexes [14–18].

Hydrazones are members of the Schiff bases family, which are built with aromatic acid hydrazides and carbonyl compounds. They are quite interesting in coordination chemistry as they present a combination of donor sites, such as a protonated/deprotonated amide oxygen atom, imine nitrogen atom of the hydrazone moiety and an additional donor site (usually N or O) provided from the aldehyde or ketone [19,20]. Hydrazones form wide variety of complexes with chemical, structural, biological and industrial importance [21–23]. These proprieties are attributed to the formation of stable chelate complexes with transition metals which catalyze physiological processes [24,25].

In this study, we report the synthesis of six new hydrazone derivatives containing pyrazole moiety with a good yield (Scheme 1). The X-ray crystal structures of compounds L1 and L2 were determined, and their geometrical parameters were compared with theoretical DFT calculations at the B3LYP level of theory. The investigation of catalytic activities of copper (II)-ligand complexes towards oxidation of catechol to o-quinone was studied. All of the parameters that can affect the catalytic efficiency were studied.

Scheme 1. The synthetic routes of compounds L1–L6: (a) hydrazine hydrate (80%), ethanol, reflux 5 h; and (b) ethanol, acetic acid, reflux, 2–5 h.
2. Results

2.1. Synthesis

Synthesis of hydrazones (L1–L6) is outlined in Scheme 1. Starting compound, ethyl 3-phenyl-1H-pyrazole-5-carboxylate (1), was readily synthesized by the reaction of ethyl-2,4-dioxo-4-phenyl-butan-2-one, obtained from acetophenone, and diethyl oxalate, with hydrazine in the presence of sulfuric acid at room temperature. The reaction of ethyl 3-phenyl-1H-pyrazole-5-carboxylate (1) with hydrazine hydrate in ethanol afforded 3-phenyl-1H-pyrazole-5-carbohydrazide (2). Finally, the novel desired hydrazones (L1–L6) were obtained by condensing compound (2) with aromatic aldehydes at reflux of ethanol using acetic acid as reported in our previous procedure [26–28]. The expected products were isolated as crystalline materials with reliable to excellent yields.

2.2. X-Ray Crystal Structures Description

Compounds L1 and L2 were analyzed by X-ray diffraction. Refinement parameters and crystal data are listed in Table S1. Supplementary data are deposited with the Cambridge Crystallographic Data Centre (CCDC) under deposition numbers 1522882 and 1523265.

The selected bond lengths and bond angles and hydrogen bonds are listed in Tables S2–S5 in the Supplementary Materials. The asymmetric unit of L1 contains one independent molecule with DMF and water molecules as mixed crystallizing solvent, as shown in Figure 1. All the bond lengths and angles are in normal ranges. In the crystal wrap, molecules are connected via many classical and non-classical intermolecular hydrogen bonds (Table S3). Figure 2 shows the crystal structure of L2. In the crystal structure, the pyrazole ring (N1/N2/C7–C9) makes dihedral angles with the phenyl ring (C1–C6) and tolyl ring (C12–C17), at 23.39° and 36.16°, respectively. In the crystal wrap, molecules are linked via two classical intermolecular hydrogen bonds between N1—H1N1···O1i and N3—H1N3···N2ii, Symmetry codes: (i) x, −y+1, z+1/2; and (ii) −x+1, y, −z+3/2 (Table S5).

![Figure 1. Asymmetric unit of L1 (CCDC 1522882).](image-url)
2.3. Computational Studies

Comparison of the theoretical values with the experimental ones indicates that all the optimized bond lengths are closer to the experimental values. In the case of X-ray structure of compound L1, the observed bond lengths of C1–C10, O3–C15 and N3–N4 bonds in five-membered pyrazole ring are 1.227(3) Å, 1.363(3) Å and 1.383(3) Å, respectively. The calculated bond lengths, through DFT method, of same pyrazole ring are 1.24394 Å, 1.38125 Å and 1.37144 Å, respectively, which are very close to the actual values. In Table 1, it is clear that actual C-C and C-H bond lengths are also in close agreement with calculated values. The calculated bond angles for O1–C10–C9, C14–O2–C18 and N4–C11–C12 bond angles of L1 are 122.22°, 118.52°, 121.37°, respectively, which are close to the corresponding actual angles obtained from X-ray. The actual values of above bond angles are 121.3°, 118.3°, and 122.9°, respectively.

| Bond Length (Å) | Experimental Bond Lengths | Calculated Bond Lengths | Bond Angle (°) | Experimental Bond Angles | Calculated Bond Angles |
|----------------|---------------------------|-------------------------|----------------|--------------------------|-----------------------|
| O1–C10        | 1.22(3)                   | 1.24                    | N2–C9–C8      | 106.0(2)                 | 109.2                 |
| O2–C14        | 1.36(3)                   | 1.39                    | O1–C10–N3     | 122.8(2)                 | 125.0                 |
| O2–C18        | 1.41(4)                   | 1.45                    | N3–C10–C9     | 116.0(2)                 | 112.7                 |
| O3–C15        | 1.36(3)                   | 1.38                    | O1–C10–C9     | 121.3(2)                 | 122.2                 |
| N1–N2         | 1.33(3)                   | 1.37                    | N4–C11–C12    | 122.9(2)                 | 121.3                 |
| N1–C7         | 1.33(3)                   | 1.38                    | O2–C14–C13    | 125.7(2)                 | 126.0                 |
| N2–C9         | 1.34(3)                   | 1.36                    | C14–O2–C18    | 118.3(2)                 | 118.5                 |
| N3–N4         | 1.38(3)                   | 1.37                    | N2–N1–C7      | 105.3(2)                 | 113.2                 |
| N3–C10        | 1.34(3)                   | 1.38                    | N1–N2–C9      | 112.6(2)                 | 105.0                 |
| N4–C11        | 1.27(3)                   | 1.29                    | N4–N3–C10     | 119.5(2)                 | 121.0                 |

The optimized geometry of compounds L1 and L2 were obtained at B3LYP/6-31G* level. Some optimized geometric parameters are also listed in Figure 3, and Tables 2 and 3.
The total energy, energy of HOMO and energy of LUMO, as well as other parameters for structures L1 and L2 are obtained theoretically and listed in Table 3. The HOMO and LUMO electron density distributions of L1 and L2 are given in Figures 4 and 5, respectively. After the analysis of the theoretical results obtained, we can say that molecules L1 and L2 have a non-planar structure. DFT calculation gives an idea about the substance reactivity and site selectivity of the frameworks. EHOMO; ELUMO, which clarifies the inevitable charge exchange collaboration inside the studied material; electronegativity (χ); hardness (η); potential (µ); electrophilicity (ω); softness (S); and softness (σ) are recorded in Table 3. The significance of η and σ is to evaluate both the reactivity and stability.
The analysis of the wave function indicates that the energy space between the molecular orbit HOMO and LUMO determines the chemical stability and the electrical transport properties of the molecule. The red and green colors of the molecular orbital ridge, respectively, represent the positive and negative phases.
The HOMO of L1 shows the charge density localized on the 4-hydroxy-3-methoxybenzaldehyde ring, while LUMO is characterized by a charge distribution on the hydrazone function, indicating that this moiety can influence the electron transition. The HOMO of L2 has a localized charge density on the pyrazole and hydrazone function, but LUMO is characterized by a charge distribution on the 4-methylbenzaldehyde ring and the hydrazone function. The energy difference between HOMO and LUMO of L1 and L2 is about 4.38 and 5.75 eV, respectively.

The energy of the smaller band space increases the stability of the molecule. The molecular boundary orbitals of L1 and L2 (HOMO–LUMO) are shown in Figures 4 and 5, respectively.

2.4. Catecholase Activity: Spectrophotometric Study

Catechol oxidation reaction catalyzed by copper complexes with ligands L1–L6 is followed by the evolution of o-quinone absorbance measured at 390 nm with a UV-vis spectrometer (Scheme 2).

![Scheme 2. Catecholase reaction.](image)

The catalytic oxidation rate variations from one complex to another are shown in Figures S1–S5. On the other hand, catechol oxidation rates were calculated and collected in Table 4. According to these results, we find that all copper complexes, formed in situ from ligands L1–L6 and salts of copper, catalyze the oxidation reaction of catechol to o-quinone, but with different rates. The catalytic activities depend highly on the ligand concentration, the nature of solvent and the type of inorganic anion (Figures S1–S5).

| Ligand/Metallic Salt | Cu(NO₃)₂ | CuCl₂ | Cu(CH₃COO)₂ | CuSO₄ |
|----------------------|---------|-------|-------------|-------|
| L1                   | 10.57   | 10.28 | 9.80        | 9.27  |
| L2                   | 15.52   | 0.05  | 22.92       | 16.06 |
| L3                   | 37.89   | 9.19  | 24.58       | 21.01 |
| L4                   | 17.71   | 11.43 | 15.02       | 6.57  |
| L5                   | 3.10    | 8.07  | 19.62       | 10.61 |
| L6                   | 27.77   | 40.27 | 60.50       | 72.92 |

It appears plainly that catalytic activity varies from one complex to another. All of the copper complexes formed by the hydrazone derivatives catalyze the oxidation reaction of catechol to o-quinone. The best is the L6 ligand with highest oxidation rate for all copper salts with maximum reaction rate equal to 72.92 µmol·L⁻¹·min⁻¹ for CuSO₄ followed by L3 with maximum rate of 37.89 µmol·L⁻¹·min⁻¹ for Cu(NO₃)₂. Lowest recorded oxidation rates, whose values are less than 10 µmol·L⁻¹·min⁻¹, correspond to complex L2-CuCl₂, followed by L5-(CuNO₃)₂ and L4-CuSO₄, and then L5-CuCl₂. The L1 ligand seems to have nearly the same activity for all copper salts determined at about 10 µmol·L⁻¹·min⁻¹.

Basically, for any ligand used except L6, Cu(CH₃COO)₂ gives highest activities and CuCl₂ gives lowest activities (Figure 6). Concerning CH₃COO⁻ and ligands L3, L4 and L6, the values of absorbance increase at the beginning of the reaction and then remain constant after a certain time. This has been
explained in previous studies by a precipitation of the complex that can take place during this decrease in absorbance.

2.4.1. Effect of Ligand Concentration on the Catecholase Activity

The effect of ligand concentration on catecholase activity is studied by varying the ratio of equivalent ligand L6: metallic salt Cu(CH₃COO)₂. Three tests were carried out in ratios: 1:1, 2:1 and 1:2. The results obtained show that the test with the ratio L6: Cu(CH₃COO)₂ = 1:2 leads to higher oxidation rate value. Therefore, the L6 complex that contains two cupric ion Cu(II) has a better catalytic activity. However, ratios 2:1 and 1:1 lead to lower oxidation rate value, and ratio 2:1 exhibits maximum at 35 min, and after slightly decreases, probably due to complex precipitation (Figure 7).

![Figure 6. Catechol oxidation in the presence of copper complexes formed with L6.](image)

![Figure 7. Catechol oxidation in methanol, in presence of formed L6 copper complexes with different concentrations.](image)
2.4.2. Solvent Effect

The effect of three solvents (MeOH, CH$_3$CN and DMF) on the oxidation reaction with the ligand L6 and the Cu(CH$_3$COO)$_2$ salt is studied in similar thermodynamic conditions. The results of that study are presented by the rate values obtained for the oxidation reaction (Figure 8). Using a polar protic solvent such as methanol promotes oxidation reaction much better than the other two aprotic solvents, i.e., acetonitrile and DMF. Our results seem to be in perfect agreement with previous studies and show that solvatation of copper by the aprotic and polar solvents such as DMF and CH$_3$CN decreases the catalytic activity of the metal cation in the oxidation reaction of catechol to $o$-quinone.

![Figure 8](image-url). Catechol oxidation in different solvents and in the presence of formed L6 copper complexes (1 Equivalent of L6 for 1 Equivalent of Cu(CH$_3$COOH)$_2$).

2.4.3. Comparison with Alternative Catalysts

Table 5 shows the catalytic activity by other catalysts reported in the literature. It is clear that the hydrazone–pyrazole derivatives, in particular ligand L6, described in this work present further improvement and show better values and higher activity for the effective aerobic oxidation of the catechol into $o$-quinone.

| Cu(II)-Ligands     | Cu(II) Salt Used | Oxidation Rate (µmol·L$^{-1}$·min$^{-1}$) | Ref. |
|--------------------|-----------------|---------------------------------|------|
| ligand L6          | CuSO$_4$        | 72.920                          | -    |
| ligand L6          | Cu(CH$_3$COO)$_2$ | 60.500                          | -    |
| ligand L6          | CuCl$_2$        | 40.270                          | -    |
| C,N-bipyrazole     | Cu(CH$_3$COO)$_2$ | 4.440                           | [29] |
| bipyrzalic         | Cu(CH$_3$COO)$_2$ | 11.825                          | [30] |
| tripode-prop-2-yacetate | CuCl$_2$     | 1.458                           | [31] |
| bipyrzalic         | CuSO$_4$        | 28.990                          | [32] |
| tripode-4-hydroxyphenyl | CuCl$_2$   | 4.378                           | [33] |
| bipyrzalic         | Cu(CH$_3$COO)$_2$ | 31.780                          | [34] |
| tripode-3-hydroxypropyl | CuSO$_4$   | 8.710                           | [35] |
The superior catalytic activity observed for ligand L6 is probably due to the stability of the corresponding copper complex (catalyst) favored by the organic conjugate π bonds of the three benzene ring contained in the ligand and by the intense coordination bonds of the Schiff base. To our knowledge, the catalytic activity observed for ligand L6 (72.920 µmol·L⁻¹·min⁻¹) is the most important among the catalysts described in the literature.

3. Experimental

3.1. General Methods

The chemical reagents used in synthesis were purchased from Merck, Darmstadt, Germany or Aldrich, St. Louis, MO, USA; were of the highest commercially available purity; and were used without previous purification. Melting points were measured using a Büchi B-545 digital capillary melting point apparatus (BUCHI Labortechnik AG, Flawil, CH, Switzerland) and used without correction. Reactions were checked with TLC silica gel 60 F254 (MACHEREY-NAGEL, Neumann-Neander, Drun, Germany). Spectra IR were recorded on a VERTEX 70 FT-IR spectrometer (Perkin-Elmer, Billerica, MA, USA) and frequencies are reported in cm⁻¹. The spectra of ¹H NMR and ¹³C NMR were recorded in solution in DMSO-d₆ on a Bruker Avance 300 NMR spectrometer (Bruker, MA, USA). The chemical shifts are expressed in parts per million (ppm) by using tetramethylsilane (TMS) as internal reference and coupling constants (J) are given in Hz. Mass spectra were collected using a API 3200 LC/MS/MS system (AB MDS Sciex, Ontario, Canada) equipped with an ESI source. DRX data were collected on a Bruker APEX-II D8 Venture area diffractometer (Bruker, MA, USA) equipped with graphite monochromatic Mo Kα radiation, λ = 0.71073 Å at 293 (2) and 296 (2) K, respectively. The electronic spectra of the ligand and its metal complexes were measured on a Lambda 35 ES UV/VIS spectrophotometer (Perkin Elmer, Waltham, MA, USA) in the range of 200–900 nm.

3.2. Synthesis

3.2.1. Synthesis of 3-phenyl-1H-pyrazole-4-carbohydrazides (2)

To a stirred solution of 1 mmol of the 3-phenyl-1H-pyrazole-4-carboxylate (1) in ethanol (10 mL), 2 mL of 80% hydrazine monohydrate was added. The reaction mixture was maintained under reflux for 5 h, until TLC indicated the end of reaction. Afterwards, the reaction mixture was poured onto ice and the solid formed was collected by filtration, washed with cold water and recrystallized from ethanol. Yield: 66%; m.p: 207–209 ºC; IR (ν(cm⁻¹)): 3296–3203 (NH, NH₂), 1629 (C=O); ¹H NMR: (300 MHz, DMSO-d₆, δ(ppm)): 4.45 (s, 2H, NH₂), 7.05 (1H, s, CH-pyrazole), 7.17–7.60 (5H, m, Ar-H), 9.38 (s, 1H, NHCO), 13.61 (1H, s, NH-pyrazole); ESI-MS: m/z = 203.3 [M+H]+, 225.1 [M+Na]+. The FT-IR, ¹H NMR and mass spectra of compound 2 are shown in Figures S6–S8.

3.2.2. General Procedure for the Synthesis of Ligands (L1–L6)

To a solution of 5-phenyl-1H-pyrazole-4-carboxylic acid (2) (1 mmol) in 10 mL of ethanol, an equimolar amount of the appropriate benzaldehyde derivative was added in the presence of acetic acid. The mixture was maintained under reflux for 2–5 h, until TLC indicated the end of reaction. Then, the reaction mixture was cooled to 25 ºC, and the precipitate formed was filtered out washed with ethanol and recrystallized from ethanol. The FT-IR, ¹H, ¹³C NMR and mass spectra of Ligands (L1–L6) are shown in Figures S9–S30.

3.2.3. N’-(4-hydroxy-3-methoxybenzylidene)-5-phenyl-1H-pyrazole-3-carbohydrazide (L1)

Yield: 85%; m.p: 229–231 ºC; IR (ν(cm⁻¹)): 3276 (NH), 1681 (C=O), 1568 (C=N); ¹H NMR: (300 MHz, DMSO-d₆, δ(ppm)): 3.81 (3H, s, -OCH₃), 6.83 (d, J = 8.1 Hz, 1H, H-Ar), 7.18 (1H, s, CH-pyrazole), 7.26–7.49 (5H, m, Ar-H), 7.54 (s, 1H, H-Ar), 7.82 (d, J = 8.1 Hz, 1H, H-Ar), 8.39 (1H, s, N=CH), 11.51 (s, 1H, OH), 11.72 (s, 1H, NHCO), 13.72 (1H, s, NH-pyrazole); ¹³C NMR: (300 MHz,
DMSO-d_6, δ (ppm)): 56.04 (OCH_3), 103.85 (CH, C_4-pyrazole), 115.91 (CH, C-Ar), 122.53 (CH, C-Ar), 125.53 (CH, C-Ar), 126.37 (CH, C-Ar), 128.28 (CH, C-Ar), 129.07 (CH, C-Ar), 129.33 (C, C-Ar), 144.12 (C, N=CH), 145.85 (C, C_3-pyrazole), 150.36 (C, C_5-pyrazole), 156.48 (C, C-OCH_3), 158.40 (C, C=O). ESI-MS: m/z = 337.0 [M+H]^+, 359.0 [M+Na]^+.

3.2.4. N’-(4-methylbenzylidene)-5-phenyl-1H-pyrazole-3-carboxydrazide (L2)

Yield: 90%; m.p: 297–299 ºC; IR (ν (cm⁻¹)): 3205 (NH), 1680 (C=O), 1561 (C=N); ¹H NMR: (300 MHz, DMSO-d_6, δ (ppm)): 2.32 (3H, s, CH_3), 7.25 (1H, s, CH-pyrazole), 7.35–7.61 (5H, m, Ar-H), 7.59 (d, J = 7.8 Hz, 2H, H-Ar), 7.61 (d, J = 7.8 Hz, 1H, H-Ar), 8.45 (1H, s, N=CH), 11.65 (s, 1H, NHCO), 13.78 (1H, s, NH-pyrazole); ¹³C NMR: (300 MHz, DMSO-d_6, δ (ppm)): 31.50 (OCH_3), 103.85 (CH, C_4-pyrazole), 125.80 (CH, C-Ar), 127.53 (CH, C-Ar), 127.90 (CH, C-Ar), 128.94 (CH, C-Ar), 129.49 (CH, C-Ar), 129.92 (C, C-Ar), 132.18 (C, C-Ar), 136.80 (C, C_3-pyrazole), 146.18 (CH, N=CH), 148.28 (C, C_5-pyrazole), 158.50 (C, C=O). ESI-MS: m/z = 304.9 [M+H]^+, 326.9 [M+Na]^+.

3.2.5. N’-(4-chlorobenzylidene)-5-phenyl-1H-pyrazole-3-carboxydrazide (L3)

Yield: 89%; m.p: 301–303 ºC; IR (ν (cm⁻¹)): 3207 (NH), 1680 (C=O), 1605 (C=N); ¹H NMR: (300 MHz, DMSO-d_6, δ (ppm)): 7.11 (1H, s, CH-pyrazole), 7.33–7.60 (5H, m, Ar-H), 7.59 (d, J = 7.2 Hz, 2H, H-Ar), 7.81 (d, J = 7.2 Hz, 1H, H-Ar), 8.46 (1H, s, N=CH), 11.65 (s, 1H, NHCO), 13.79 (1H, s, NH-pyrazole); ¹³C NMR: (300 MHz, DMSO-d_6, δ (ppm)): 103.82 (CH, C_4-pyrazole), 125.82 (CH, C-Ar), 127.53 (CH, C-Ar), 127.90 (CH, C-Ar), 129.49 (CH, C-Ar), 129.91 (C, C-Ar), 132.18 (C, C-Ar), 140.28 (C, C_3-pyrazole), 156.88 (CH, N=CH), 158.62 (C, C_5-pyrazole), 164.13 (C, C=O). ESI-MS: m/z = 325.1 [M+H]^+, 347.3 [M+Na]^+.

3.2.6. N’-(4-fluorobenzylidene)-5-phenyl-1H-pyrazole-3-carboxydrazide (L4)

Yield: 98%; m.p: 294–296 ºC; IR (ν (cm⁻¹)): 3320 (NH), 1672 (C=O), 1604 (C=N); ¹H NMR: (300 MHz, DMSO-d_6, δ (ppm)): 7.21 (1H, s, CH-pyrazole), 7.25–7.38 (5H, m, Ar-H), 7.42 (d, J = 7.8 Hz, 2H, H-Ar), 7.81 (d, J = 7.8 Hz, 1H, H-Ar), 8.50 (1H, s, N=CH), 11.72 (s, 1H, NHCO), 13.79 (1H, s, NH-pyrazole); ¹³C NMR: (300 MHz, DMSO-d_6, δ (ppm)): 103.86 (CH, C_4-pyrazole), 125.82 (CH, C-Ar), 128.98 (CH, C-Ar), 129.49 (CH, C-Ar), 129.64 (C, C-Ar), 129.74 (CH, C-Ar), 130.10 (C, C-Ar), 130.22 (C, C-Ar), 146.97 (C, C_3-pyrazole), 150.50 (CH, N=CH), 156.68 (C, C_5-pyrazole), 161.90 (C, C=O), 165.18 (C, C-F). ESI-MS: m/z = 309.3 [M+H]^+.

3.2.7. 5-phenyl-N’-(1-phenylethylidene)-1H-pyrazole-3-carboxydrazide (L5)

Yield: 85%; m.p: 251–253 ºC; IR (ν (cm⁻¹)): 3320 (NH), 1667 (C=O), 1589 (C=N); ¹H NMR: (300 MHz, DMSO-d_6, δ (ppm)): 2.36 (3H, s, CH_3), 7.24 (1H, s, CH-pyrazole), 7.49–7.84 (10H, m, Ar-H), 10.37 (s, 1H, NHCO), 13.82 (1H, s, NH-pyrazole); ¹³C NMR: (300 MHz, DMSO-d_6, δ (ppm)): 21.51 (OCH_3), 103.60 (CH, C_4-pyrazole), 125.73 (CH, C-Ar), 127.72 (CH, C-Ar), 128.70 (CH, C-Ar), 129.01 (CH, C-Ar), 129.18 (CH, C-Ar), 129.56 (CH, C-Ar), 130.34 (C, C-Ar), 130.07 (C, C-Ar), 137.21 (C, C_3-pyrazole), 144.82 (CH, N=CH), 146.45 (C, C_5-pyrazole), 157.23 (C, C=O). ESI-MS: m/z = 305.4 [M+H]^+.

3.2.8. N’-(diphenymethylene)-5-phenyl-1H-pyrazole-3-carboxydrazide (L6)

Yield: 82%; m.p: 200–202 ºC; IR (ν (cm⁻¹)): 3360 (NH), 1664 (C=O), 1537 (C=N); ¹H NMR: (300 MHz, DMSO-d_6, δ (ppm)): 7.20 (1H, s, CH-pyrazole), 7.32–7.76 (15H, m, Ar-H), 8.69 (s, 1H, NHCO), 13.84 (1H, s, NH-pyrazole); ¹³C NMR: (300 MHz, DMSO-d_6, δ (ppm)): 105.10 (CH, C_4-pyrazole), 127.66 (CH, C-Ar), 128.66 (CH, C-Ar), 128.96 (CH, C-Ar), 130.21 (CH, C-Ar), 130.31 (CH, C-Ar), 130.46 (CH, C-Ar), 132.02 (C, C-Ar), 132.14 (C, C-Ar), 140.08 (C, C_3-pyrazole), 145.70 (CH, N=CH), 153.35 (C, C_5-pyrazole), 157.62 (C, C=O). ESI-MS: m/z = 366.9 [M+H]^+, 389.0 [M+H]^+.
3.3. X-Ray Crystallographic Analysis

The compounds of L1 and L2 were obtained as single crystals by slow evaporation from ethanol solution of the pure compound at room temperature. Cell refinement and data reduction were carried out by Bruker SAINT. SHELXT was used to solve structure [36,37]. The final refinement was carried out by full-matrix least-squares techniques with anisotropic thermal data for no hydrogen atoms on F.

CCDC 1522882 and 1523265 for L1 and L2, respectively, contain the supplementary crystallographic data for these compounds can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

3.4. DFT Computational Method

The computational studies of compounds L1 and L2 were performed at the B3LYP/6-31G level of theory using Gaussian 09 package programs [38,39]. The optimization geometries of L1 and L2 were performed using the Berny analytical gradient optimization method [40].

3.5. Catecholase Activity Measurement

Kinetic measurements were made spectrophotometrically on UV-vis spectrometer, following the appearance of o-quinone over time at 25 °C (390 nm absorbance maximum ε = 1600 L mol⁻¹ cm⁻¹ in methanol [35]. The complexes were prepared in situ by successively mixing 0.15 mL of a solution (2 × 10⁻³ M) of CuX₂·nH₂O (X = Cl⁻, NO₃⁻, CH₃COO⁻ or SO₄²⁻), with 0.15 mL of a solution (2 × 10⁻³ M) of ligand, then adding 2 mL of a solution of catechol at a concentration of 10⁻¹ M.

4. Conclusions

In this work, we report the synthesis of six new hydrazone–pyrazole biomolecules (L1–L6) in excellent yields. The X-ray structures of L1 and L2 have been investigated herein for the first time. The theoretical calculations through DFT of L1 and L2 well supported the experimental findings. These ligands (L1–L6) and different Cu(II) salts demonstrate an efficient activity to catalyze the aerobic oxidation of the catechol into o-quinone compared to others recent catalysts described in the literatures. Interestingly, ligand L6 exhibits an extremely high rate of oxidation, attaining 72.92 µmol·L⁻¹·min⁻¹, which is, to our knowledge, the best catalytic activity among the reported catalysts. Cu(II)-ligand complexes were generated in situ and the results obtained show that the oxidation depend highly on several parameters: the nature and concentration of the ligand, the nature of salts and the solvent effects. The results suggest that these new materials have potential for the oxidation of the catechol into o-quinone, thus opening important perspectives.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/1422-0067/18/11/2215/s1.

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References
1. Hartinger, C.G.; Dyson, P.J. Bioorganometallic chemistry—from teaching paradigms to medicinal applications. Chem. Soc. Rev. 2009, 38, 391–401. [CrossRef] [PubMed]
2. Patra, M.; Gasser, G. Organometallic Compounds: An Opportunity for Chemical Biology? ChemBioChem 2012, 13, 1232–1252. [CrossRef] [PubMed]
3. Daumann, L.J.; Schenk, G.; Ollis, D.L.; Gahan, L.R. Spectroscopic and mechanistic studies of dinuclear metallohydrodolases and their biomimetic complexes. *Dalton Trans.* 2014, 43, 910–928. [CrossRef] [PubMed]

4. Dalle, K.E.; Meyer, F. Modelling binuclear metallobiotics: Insights from pyrazole-supported biomimetic and bioinspired complexes. *Eur. J. Inorg. Chem.* 2015, 3391–3405. [CrossRef]

5. Mistri, S.; Paul, A.; Bhunia, A.; Manne, R.K.; Santra, M.K.; Puschmann, H.; Manna, S.C. A combined experimental and theoretical investigation on the Cu(II) sensing behavior of a piperazine moiety based ligand, and catecholase and biological activities of its Cu(II) complex in combination with pyridine 2,5-dicarboxylate. *Polyhedron* 2016, 104, 63–72. [CrossRef]

6. Rosenzweig, A.C.; Szajnski, M.H. Structural insights into dioxygen-activating copper enzymes. *Curr. Opin. Struct. Biol.* 2006, 16, 729–735. [CrossRef] [PubMed]

7. Mirica, L.M.; Ottenwaelder, X.; Stack, T.D.P. Structure and Spectroscopy of Copper–Dioxygen Complexes. *Chem. Rev.* 2004, 104, 1013–1046. [CrossRef] [PubMed]

8. Itoh, S.; Fukuzumi, S. Monooxygenase Activity of Type 3 Copper Proteins. *Acc. Chem. Res.* 2007, 40, 592–600. [CrossRef] [PubMed]

9. Mutti, F.G.; Zoppellaro, G.; Gullotti, M.; Santagostini, L.; Pagliarin, R.; Andersson, K.K.; Casella, L. Biomimetic Modelling of Copper Enzymes: Synthesis, Characterization, EPR Analysis and Enantioselective Catalytic Oxidations by a New Chiral Trinuclear Copper(II) Complex. *Eur. J. Inorg. Chem.* 2009, 2009, 554–566. [CrossRef]

10. Balasubramanian, V.; Ezhevskaya, M.; Moons, H.; Neuberger, M.; Cristescu, C.; Van Doorslaer, S.; Palivan, C. Structural characterization of a highly active superoxide-dismutase mimic. *Phys. Chem. Chem. Phys.* 2009, 11, 6778–6787. [CrossRef] [PubMed]

11. Sreenivasulu, B. Diphenoxo-Bridged Copper(II) Complexes of Reduced Schiff Base Ligands as Functional Models for Catechol Oxidase. *Aust. J. Chem.* 2009, 62, 968–979. [CrossRef]

12. Solomon, E.I.; Sundaram, U.M.; Machonkin, T.E. Multicopper Oxidases and Oxygenases. *Chem. Rev.* 1996, 96, 2563–2606. [CrossRef] [PubMed]

13. Eicken, C.; Krebs, B.; Sacchettini, J.C. Catechol oxidase—structure and activity. *Curr. Opin. Struct. Biol.* 1999, 9, 677–683. [CrossRef]

14. Beyazit, N.; Çatıkka, B.; Bayraktar, Ş.; Demetgül, C. Synthesis, characterization and catecholase-like activity of new Schiff base metal complexes derived from visnagin: Theoretical and experimental study. *J. Mol. Struct.* 2016, 1119, 124–132. [CrossRef]

15. Shaban, S.Y.; Ramadan, A.E.-M.M.; Ibrahim, M.M.; Mohamed, M.A.; van Eldik, R. Spectroscopic, thermodynamic, kinetic studies and oxidase/antioxidant biomimetic catalytic activities of tris(3,5-dimethylpyrazolyl)borate Cu(II) complexes. *Dalton Trans.* 2015, 44, 14110–14121. [CrossRef] [PubMed]

16. Comba, P.; Martin, B.; Muruganamthan, A.; Straub, J. Structure, Bonding, and Catecholase Mechanism of Copper Bispidine Complexes. *Inorg. Chem.* 2012, 51, 9214–9225. [CrossRef] [PubMed]

17. Ackermannna, J.; Buchlerb, S.; Meyer, F. Structure–activity correlations in highly preorganized dicopper catechol oxidase model systems. *C. R. Chim.* 2007, 10, 421–432. [CrossRef]

18. Belle, C.; Selmeczi, K.; Torelli, S.; Pierre, J.-L. Chemical tools for mechanistic studies related to catechol oxidase activity. *C. R. Chim.* 2007, 10, 271–283. [CrossRef]

19. Matoga, D.; Szklarzewicz, J.; Stadnicka, K.; Shongwe, M.S. Iron (III) complexes with a biologically relevant aryldihydrazone: crystallographic evidence for coordination versatility. *Inorg. Chem.* 2007, 46, 9042–9044. [CrossRef] [PubMed]

20. Shongwe, M.S.; Al-Rahbi, S.H.; Al-Azani, M.A.; Al-Muhabari, A.A.; Al-Mjeni, F.; Matoga, D.; Gismelseed, A.; Al-Omari, I.A.; Yousif, A.; Adams, H.; et al. Coordination versatility of tridentate pyridyl aryldihydrazone towards iron: tracking down the elusive aryldihydrazone-based ferric spin-crossover molecular materials. *Dalton Trans.* 2012, 41, 2500–2514. [CrossRef] [PubMed]

21. Anbu, S.; Paul, A.; Ribeiro, A.P.C.; da Silva, M.F.C.G.; Kuznetsov, M.S.L.; Pombeiro, A.J.L. Biomolecular interaction, catecholase like activity and alkane oxidation in ionic liquids of a phenylcarbohydrazone-based monocopper(II) complex. *Inorg. Chim. Acta* 2016, 450, 426–436. [CrossRef]

22. Ruben, M.; Leh, J.-M.; Vaughan, G. Synthesis of ionisable [2 × 2] grid-type metallo-arrays and reversible protonic modulation of the optical properties of the [CoIII4L4]8+ species. *Chem. Commun.* 2003, 12, 1338–1339. [CrossRef]
23. Mondal, S.; Pakhira, B.; Blake, A.J.; Drew, M.G.B.; Chattopadhyay, S.K. Co(III) and Ni(II) complexes of an anthracene appended aroyl hydrazone: Synthesis, crystal structures, DNA binding and catecholase activity. *Polyhedron* 2016, 117, 327–337. [CrossRef]

24. Katyal, M.; Dutt, Y. Analytical applications of hydrazones. *Talanta* 1975, 22, 151–166. [CrossRef]

25. Mohan, M.; Gupta, M.P.; Chandra, L.; Jha, N.K. Synthesis, characterization and antitumour properties of some metal (II) complexes of 2-pyridinecarboxaldehyde 2′-pyridylhydrazone and related compounds. *Inorg. Chim. Acta* 1988, 151, 61–68. [CrossRef]

26. Karrouchi, K.; Charkaoui, Y.; Benlafya, K.; Ramli, Y.; Taoufik, J.; Radi, S.; Ansar, M. Synthesis, characterization and preliminary biological activity of some new pyrazole carbohydrazide derivatives. *J. Chem. Pharm. Res.* 2013, 5, 1–6.

27. Karrouchi, K.; Radi, S.; Taoufik, J.; Ghabbour, H.A.; Mabkhot, Y.N. Crystal structure of N′-(4-nitrobenzylidene)-5-phenyl-1H-pyrazole-3-carbohydrazide, C17H13N5O3. *Z. Krist. New Cryst. Struct.* 2016, 231, 839–841. [CrossRef]

28. Karrouchi, K.; Ansar, M.; Radi, S.; Saadi, M.; El Ammari, L. Crystal structure of N′-diphenylmethylidene-5-methyl-1H-pyrazole-3-carbohydrazide. *Acta Crystallogr. E Crystallogr. Commun.* 2015, 71, o890–o891. [CrossRef] [PubMed]

29. El Kodadi, M.; Malek, F.; Touzani, R.; Ramdani, A. Synthesis of new tripodal ligand 5-(bis(3,5-dimethyl-1H-pyrazol-1-ylmethyl)amino)pentan-1-ol, catecholase activities studies of three functional tripodal pyrazolyl N-donor ligands, with different copper (II) salts. *Catal. Commun.* 2008, 9, 966–969. [CrossRef]

30. Boussaleh, N.; Touzani, R.; Bouabdallah, I.; El Kadiri, S.; Ghalem, S. Synthesis, structure and catalytic properties of tripodal amino-acid derivatized pyrazole-based ligands. *J. Mol. Catal. A Chem.* 2009, 306, 113–117. [CrossRef]

31. Bouabdallah, I.; Touzani, R.; Zidane, I.; Ramdani, A. Synthesis of new tripodal ligand: N,N-bis[(1,5-dimethylpyrazol-3-yl)methyl]benzylamine.: Catecholase activity of two series of tripodal ligands with some copper (II) salts. *Catal. Commun.* 2007, 8, 707–712. [CrossRef]

32. Zerrouki, A.; Touzani, R.; El Kadiri, S. Synthesis of new derivatized pyrazole based ligands and their catecholase activity studies. *Arab. J. Chem.* 2011, 4, 459–464. [CrossRef]

33. Mouadili, A.; Attayibat, A.; El Kadiri, S.; Radi, S.; Touzani, R. Catecholase activity investigations using in situ copper complexes with pyrazole and pyridine based ligands. *Appl. Catal. A Gen.* 2013, 454, 93–99. [CrossRef]

34. Thabti, S.; Djedouani, A.; Rahmouni, S.; Touzani, R.; Bendaa, A.; Mousser, H.; Mousser, A. Synthesis, X-ray crystal structures and catecholase activity investigation of new chalcone ligands. *J. Mol. Struct.* 2015, 1102, 295–301. [CrossRef]

35. Mouadili, A.; Zerrouki, A.; Herrag, L.; Hammouti, B.; El Kadiri, S.; Touzani, R. Catechol oxidation: activity studies using electron-rich nitrogen-based ligands. *Res. Chem. Intermed.* 2012, 38, 2427–2433. [CrossRef]

36. Sheldrick, G.M. A short history of SHELX. *Acta Crystallogr.* 2008, 64, 112–122. [CrossRef] [PubMed]

37. Sheldrick, G.M. Program SHELXTL97; Siemens Analytical X-ray Instruments, Inc.: Madison, WI, USA, 1997. [CrossRef]

38. Becke, A.D. Density-functional thermochemistry. III. The role of exact exchange. *Chem. Phys.* 1993, 98, 5648. [CrossRef]

39. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G.A.; et al. Gaussian 09, Revision E.01; Gaussian, Inc.: Wallingford CT, England, 2009.

40. Becke, A.D. Density-functional exchange-energy approximation with correct asymptotic behavior. *Phys. Rev.* 1988, 38, 3098. [CrossRef]

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