Research Article

Anion-Dependent Synthesis of Cu(II) Complexes with 2-(1H-Tetrazol-5-yl)-1H-indole: Synthesis, X-Ray Structures, and Radical Scavenging Activity

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Two mononuclear Cu(II) complexes, [Cu(phen)2(HL)]ClO4·H2O·2DMF (1) and [Cu(phen)2(HL)2]·EtOH (2), comprising 1,10-phenanthroline (phen) and 2-(1H-tetrazol-5-yl)-1H-indole ligand (H2L) ligands are reported. Analysis and characterization of the samples were performed using standard physicochemical techniques, elemental analysis, nuclear magnetic resonance, Fourier transform infrared spectroscopy, and UV-vis spectroscopy. Single-crystal X-ray crystallography revealed the formation of a pentacoordinate complex in 1 and a hexacoordinate complex in 2, in which the anionic ligand HL− has undergone monodentate coordination through the tetrazole unit. Furthermore, the crystal structure of H2L·MeOH is also discussed. The potential application of compounds 1 and 2 in bioinorganic chemistry was addressed by investigating their radical scavenging activity with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and the results were supported also by theoretical calculations.

1. Introduction

Reactive oxygen species (ROS) and their physiological effects have been studied extensively since their discovery circa 60 years ago [1]. These ROS can be divided into two groups: free oxygen radicals such as superoxide radical O2−, hydroxyl radical OH, or organic peroxyl radicals ROO and nonradical ROS such as ozone O3, hydrogen peroxide H2O2, and singlet oxygen 1O2 [2]. As the name suggests, these molecules are very reactive and are partly responsible for oxidative stress in cells leading to lipid peroxidation [3] and damage to DNA and proteins [4, 5]. Their effect is generally considered toxic to the body and an increased level of these species has been linked to a number of pathologies such as inflammations [6], various cardiac diseases [7], and cancer [2].

The most common superoxide radical O2− is created in the electron transport chain, specifically in complexes I and III, as an unwanted by-product. Subsequently, this radical is released into the cytosol and in lesser degree to the mitochondrial matrix [8], where it then either reacts with nonenzymatic antioxidants, such as glutathione or ascorbic acid, or transforms into less damaging hydrogen peroxide or oxygen by metal-containing enzymes known as superoxide dismutases (SODs). The most common metal ions contained in these enzymes are Cu2+, Zn2+, and Mn2+; these are also directly involved in the enzymatic reaction due to their ability to transfer the unpaired electron from the superoxide radical without forming yet another highly reactive radical [9]. Klug-Roth and Rabani et al. have shown that copper ion cycles between oxidation states (I) and (II), as shown by reactions (1) and (2), creating either hydrogen peroxide or oxygen molecule [10].

\[
\begin{align*}
\text{Cu(II)} - E + O_2^- & \rightarrow \text{Cu(I)} - E + \text{O}_2 \\
\text{Cu(I)} - E + O_2^- + 2H & \rightarrow \text{Cu(I)} - E + H_2O_2
\end{align*}
\]
To improve the health conditions of patients affected by diseases linked to the increased oxidative stress, many research groups have tried to prepare complex compounds of low molecular weight that would mimic the activity of SODs; unfortunately, SODs themselves cannot be administered, as they would not pass the cell membrane and are quickly metabolized by kidneys [11, 12]. In addition to the SOD-like activity of many copper compounds, many copper complexes are also known for their cytotoxic properties, most notably a group of ternary complexes called Casiopeinas. These copper complexes are made of substituted 1,10-phenanthroline or 2,2'-bipyridines and various anionic O,O- and N,O-ligands such as glycinate or acetylacetonate. The most prominent derivatives have been able to achieve values of IC50 in the range of low micromolar concentrations on several tumor cell lines [13, 14].

To mimic the copper coordination sphere in Cu,Zn-SOD, which consists of 4 histidine ligands bound to a copper center by imidazole nitrogen atoms [15], we have decided to use a ligand containing tetrazole, 2-(1H-tetrazol-5-yl)-1H-indole (H2L) (Scheme 1). Tetrazole is also a five-membered nitrogen-containing ring similar to that of imidazole, but tetrazoles can readily release proton and have acidic properties similar to those of carboxylic acid.

Several complexes containing tetrazole have already exhibited cytotoxic properties, most notably a dimeric Pt(II) complex prepared by Komeda et al., which was exhibited cytotoxic properties, most notably a dimeric carboxylic acid.

2. Materials and Methods

All solvents and chemicals were purchased from various commercial sources and used without further purification. Elemental analysis was performed on the Thermo Scientific Flash 2000 analyzer. The infrared spectra of the complexes were measured on a Jasco FT/IR-4700 using ATR technique with a diamond plate in the range of 400–4000 cm\(^{-1}\). The \(^1\)H, \(^13\)C, and 2D NMR spectra of the ligand were measured on a 400 MHz Varian spectrometer. UV/Vis spectra were measured on a Cintra 3030 (GBC Scientific Instruments) double beam spectrometer.

2.1. Synthesis

2.1.1. Synthesis of 2-(1H-Tetrazol-5-yl)-1H-indole. The solution of 5 g (31 mmol) of 1H-indole-2-carboxylic acid in 25 ml of chloroform was mixed with 5 ml of thionyl chloride (SOCl\(_2\)) and three drops of dimethylformamide (DMF). The reaction mixture was then refluxed for 2 hours. The cooled solution was then poured into a slurry of 20 ml of aqueous ammonia and 20 g of ice. The mixture was then stirred for 2 hours at room temperature, during which a large amount of yellow precipitate appeared. The solid product was filtered off, washed with water, and dried in a vacuum desiccator.

The product, 1H-indole-2-carboxamide, was used in the next step without further purification (yield: 4.4 g (89%)).

6.3 g (39 mmol) of the previously prepared 1H-indole-2-carboxamide was added to 50 ml of phosphor chloride (POCl\(_3\)). This mixture was refluxed for 30 minutes and subsequently it was poured onto 100 g of ice. The pH of the mixture was then adjusted to 8 by aqueous ammonia during which light brown product precipitated. This suspension was extracted 3 times with 50 ml of diethyl ether. The organic phase was dried over Na\(_2\)SO\(_4\) and filtered, and the solvent was removed to dryness on a rotatory evaporator. The light brown product, 1H-indole-2-carboxonitrile, was dried in a vacuum desiccator and used in the next step without further purification (yield: 4.12 g (74%)).

4.12 g (29 mmol) of 1H-indole-2-carbonitrile was dissolved in 25 ml of DMF. To this was then added 3.77 g (58 mmol) of Na\(_2\)S and 1.55 g (29 mmol) of NH\(_4\)Cl. This suspension was heated at 120°C for 18 hours. After it was cooled to room temperature, this mixture was poured into 100 ml of distilled water. The pH was adjusted to 1–2 with 2M HCl and the solution was extracted 3 times with 50 ml of ethyl acetate. The organic phase was washed with brine and dried over Na\(_2\)SO\(_4\). After filtration, the solvent was removed on a rotatory evaporator to dryness. The resulting brown powder was recrystallized from methanol with a spoon of activated charcoal. The resulting off-white crystals of 2-(1H-tetrazol-5-yl)-1H-indole (H2L) were suitable for X-ray diffraction analysis (yield: 2.84 g (45%)).

 Anal. Calcd. (%): for C\(_{10}\)H\(_{11}\)N\(_2\)O (M\(_r\) = 217.1) corresponding to H\(_2\)L:MeOH: C, 55.3; H, 5.1; N, 32.2. Found: C, 54.9; H, 4.9; N, 32.3. FT-IR (ATR, cm\(^{-1}\)): 438w, 525w, 709w, 742s, 801m, 892w, 923m, 1005w, 1023w, 1083m, 1118w, 1137w, 1233m, 1249w, 1277w, 1338s, 1378s, 1414w, 1455w, 1509w, 1573w, 1618s, 2640w, 2687w, 2775w, 2832w, 2894w, 2964w, 3034w, 3111w, 3223s, 3362w, 3478w. \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 7.04 (t, \(J = 7.63\) Hz, 1 H, C2-H), 7.19 (t, \(J = 7.63\) Hz, 1 H, C5-H), 7.47 (d, \(J = 8.22\) Hz, 1 H, C6-H), 7.64 (d, \(J = 8.22\) Hz, 1 H, C3-H).
12.13 (br. s., 1 H, N1-H). 13C NMR (DMSO-d6) δ (ppm): 104.05, 112.66, 120.55, 121.58, 122.38, 124.01, 127.88, 137.82, 150.48.

2.1.2. Synthesis of Compound 1. 42.7 mg (115 μmol) of Cu(CIO4)2·6H2O was dissolved in 5 ml of DMF together with 45.6 mg (230 μmol) of 1,10-phenanthroline monohydrate and 25 mg (115 μmol) of H2L. Subsequently, 115 μl of 1 M aq. NaOH solution was added to deprotonate the ligand. The formation of green crystals suitable for an XRD analysis was observed upon diethyl ether vapors diffusion into the solution. The product was filtered off, washed with diethyl ether, and dried in a vacuum desiccator (yield: 56.8 mg (56%)).

Anal. Calcd. (%) for C30H38ClCuN11O7 (M¢ = 871.8) corresponding to [Cu(phen)2(HL)]ClO4·H2O: C, 53.7; H, 4.4; N, 17.7. Found: C, 53.6; H, 4.2; N, 17.3. FT-IR (ATR, cm⁻¹): 427w, 521w, 620m, 660w, 721m, 755w, 847m, 1084s, 1145w, 1193w, 1223w, 1254w, 1308w, 1339w, 1362w, 1387w, 1424m, 1496w, 1514w, 1590w, 1625w, 1655s, 2924w, 3057w, 3345m, 3380w.

2.1.3. Synthesis of Compound 2. 46 mg (230 μmol) of Cu(OAc)2·H2O was added to 10 ml of ethanol together with 91.2 mg (460 μmol) of 1,10-phenanthroline monohydrate. After everything was dissolved, 50 mg (230 μmol) of H2L was added to the reaction mixture. The solution was mixed to complete dissolution of the ligand and the clear solution was left to evaporate at room temperature, leading to the formation of green crystals suitable for an X-ray diffraction analysis. The resulting product was filtered off, washed with diethyl ether, and dried in a vacuum desiccator (yield: 67 mg (35%)).

Anal. Calcd. (%) for C44H34CuN14O (M¢ = 838.4) corresponding to [Cu(phen)2(HL)2]: EtOH: C, 63.0; H, 4.1; N, 23.4. Found: C, 62.6; H, 3.8; N, 23.1. FT-IR (ATR, cm⁻¹): 419w, 446w, 520w, 582w, 662w, 723s, 750m, 808w, 840m, 863w, 930w, 1035w, 1100w, 1143w, 1222w, 1306w, 1335s, 1363w, 1409m, 1423m, 1496w, 1514w, 1590w, 1625w, 3057w, 3345m.

2.2. X-Ray Crystallography. The data collection for H2L·MeOH (CSD number 2114450), 1 (CSD number 2114451), and 2 (CSD number 2114452) was carried out on SuperNova diffractometer from Rigaku OD equipped with Atlas2 CCD detector and Cu Ka sealed tube as source. CrystAlisPro version 1.171.41.93a was used for the data collection and for the cell refinement, data reduction, and absorption correction [18]. The molecular structure of the prepared compounds was solved by SHELXT [19] and subsequent Fourier syntheses using SHELXL [20]. Anisotropic displacement parameters were refined for all non-H atoms. The hydrogen atoms were placed in calculated positions and refined riding on their parent C atoms with C–H (aliphatic) bond length of 0.98 Å and 0.99 Å with C–H (aromatic) bond length of 0.95 Å in all three compounds. The hydrogen atoms of hydroxyl groups were also placed in calculated positions and refined riding on their parent O atoms with O–H bond length of 0.84 Å for H2L·MeOH and 0.83 Å for H2L·H2O.

2.3. DPPH Scavenging Activity. The DPPH scavenging assay was performed with some modifications according to a method reported by L. Tabrizi et al. [22]. In a cuvette, 150 μl of 1 mM solution of DPPH in methanol was mixed with 150/300/450 μl of 0.5 mM methanolic solution of a copper complex and the volume was adjusted to 3 ml with methanol. The solution was then mixed vigorously, and the absorbance was measured at 517 nm after 30 minutes. All experiments were carried out in triplicate. The resulting concentrations of the samples prepared this way were cDPPH = 50 μM and ccomplex = 25/50/75 μM.

The radical scavenging activity was then determined by the following equation:

\[ A(\%) = \left( 1 - \frac{A}{A_0} \right) \times 100, \]

where A is the measured absorbance of the sample and A0 is the absorbance of pure DPPH.

3. Results and Discussion

3.1. Synthesis and General Characterization. First, 2-(1H-tetrazol-5-yl)-1H-indole (H2L) acting as a ligand was prepared by three-step chemical synthesis (Scheme 2), in which the 1H-indole-2-carboxylic acid was first converted to its amide using chlorination with SOCl2 and subsequent reaction with aq. ammonia. The resulting 1H-indole-2-carboxamide was dehydrated in the second step to a nitrile. The 1H-indole-2-carbonitrile was then transformed into a tetrazole derivative H2L with the help of in situ generated HN3 [23]. The formation of H2L was confirmed by the elemental analysis and 1H and 13C NMR (Figures S1 and S2). NMR spectra were compared with already published ones [24], which confirmed that synthesis of 2-(1H-tetrazol-5-yl)-1H-indole has been successful. In 1H NMR spectrum, we have additionally also observed a characteristic peak of CH3 group of methanol with a chemical shift δ = 3.14 ppm, which we at first assumed was residual solvent peak [25]. However, subsequent elemental analysis, as well as crystal structure determination, indeed showed that one molecule of methanol is present within the crystal structure, which is in accordance with the measured NMR spectra. Proton signals were then assigned with the help of COSY (Figure S3), HMBC (Figure S4), and HMQC (Figure S5) measurements. Doublets with chemical shifts δ = 7.47 and 7.64 ppm belong to protons in positions 6 and 3 (Scheme 2), respectively, whereas triplets with chemical shifts δ = 7.04 and 7.19 ppm
The coordination of the heterocycles ligands is generally reflected in different relative transmittance of in-plane C-H and N-H bending vibrations at 1655–1000 cm\(^{-1}\). Nevertheless, both complexes exhibit \(\nu(N-H)\) stretching vibrations of methanol and \(\text{H}_2\text{L}\) within 3100–3500 cm\(^{-1}\). The spectrum most likely due to its fast exchange in the solvent.

Tetrazole proton is not observed in the singlet at 12.13 ppm belong to protons in positions 4 and 5, respectively. Singlet at 7.14 ppm is attributed to a proton in position 2. Lastly, broad singlet at 12.13 ppm belongs to a proton in an NH group of the indole ring. Tetrazole proton is not observed in the spectrum most likely due to its fast exchange in the solvent.

3.2. Description of the Crystal Structures. First, the crystal structure of the ligand \(\text{H}_2\text{L-MeOH}\) is discussed (Figure 1). The prepared single crystals belong to the monoclinic crystal space group with the space group \(\text{C}2/c\) (Table 1). As the data from elementary analysis and NMR spectroscopy suggest, a molecule of methanol is indeed present in the crystal structure, forming two types of hydrogen bonds with tetrazole rings. The first type is the hydrogen bond \(\text{O}_1\cdot\cdot\cdot\text{H}_1\cdot\cdot\cdot\text{N}_3\) having bond distance \(d(\text{O}_1\cdot\cdot\cdot\text{N}_3) = 2.7952(27)\) Å (Figure 1(b)). The second type is formed between protonated tetrazole and methanol with \(d(\text{N}_1\cdot\cdot\cdot\text{O}_1) = 2.6820(22)\) Å (Figure 1(b)). There are also the intermolecular hydrogen bonds between \(\text{H}_2\text{L}\) molecules formed between indole and tetrazole units with \(d(\text{N}_5\cdot\cdot\cdot\text{N}_4) = 2.9466(23)\) Å (Figure 1(c)). The detailed information about these hydrogen bonds is listed in Table 2.

Compounds 1 crystallized in a triclinic crystal system with a space group \(\text{P} – 1\) (Table 1). The asymmetric unit of \(\text{I}\) contains a \([\text{Cu}(\text{phen})_2(\text{HL})]\) complex, perchlorate anion, two molecules of DMF, and one molecule of water. The copper atom is coordinated by two phen ligands in bidentate fashion, and the anionic \(\text{HL}^-\) ligand is coordinated through the nitrogen atom of the tetrazole unit, with the respective Cu-N distances listed in Table 3. Thus, the coordination...
number is 5 for \([\text{CuN}_5]\) chromophore, and the Addison parameter [28] is equal to 0.83, which means the coordination polyhedron is close to a trigonal bipyramid, as it is also evident in Figure 2. The shortest distance between oxygen of perchlorate anion and copper atom is \(d(\text{Cu}1 \cdots \text{O}3\text{C}) = 3.9293 \text{ Å}\), which means that the perchlorate anion is not coordinated. The cocrystallized solvents form net of the hydrogen bonds together with the indole part of the anionic ligand HL (Figure 2(b) and Table 2). Both DMF molecules form O-H- O hydrogen bonds with the following donor-acceptor distances: \(d(\text{O}1\text{W} \cdots \text{O}1\text{D}) = 2.7378 \text{ Å}\) and \(d(\text{O}1\text{W} \cdots \text{O}2\text{D}) = 2.7651 \text{ Å}\). The next hydrogen bond is formed between the oxygen of water molecule and N-H group of indole with \(d(\text{N}53 \cdots \text{O}1\text{W}) = 2.8660 \text{ Å}\). Furthermore, there is \(\pi-\pi\) stacking interaction within the crystal structure of 1 in which 1,10-phenanthroline ligands are involved and the distance between their centroids is of 3.6182 Å (Figure 2(c) and Table 4).

Crystal system of compound 2 was also triclinic with a space group \(P-1\). The asymmetric unit of 2 contains a neutral complex \([\text{Cu(phen)}_2(\text{HL})_2]\) and a molecule of ethanol.
The structure of supramolecular dimers is observed in the crystal of 

grown from the phen ligands in the presence of copper(I) and/or 

tetrazole units. Moreover, the geometry, in which the axial positions 

are occupied by nitrogen atoms of the indole ring, is stabilized by 

hydrogen bonds between nitrogen atom of the tetrazole ring and 

hydrogen atom of the second complex with the shortest distance 

between benzene and indole centroids of 3.5806 Å (Table 3 and 

Figure 3(b)). Lastly, a hydrogen bond is also present between 

nitrogen atom of the tetrazole unit and ethanol molecule with 

distance of 3.66 Å (Figure 3(c)).

### 3.3. Radical Scavenging Activity

Spectroscopic determination of DPPH radical quenching is one of 

the most widely used methods which readily and reliably provide 

information about the radical scavenging activity of studied 

compounds.

Table 1: Crystallographic data and details of structure refinement of the H$_2$L ligand and coordination compounds 1 and 2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>H$_2$L-MeOH</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical formula</strong></td>
<td>C$<em>{10}$H$</em>{11}$N$_5$O</td>
<td>C$<em>{55}$H$</em>{5}$ClCu$_{11}$O$_7$</td>
<td>C$<em>{50}$H$</em>{15}$Cu$<em>{14}$N$</em>{14}$O</td>
</tr>
<tr>
<td><strong>Formula weight</strong></td>
<td>217.24</td>
<td>871.79</td>
<td>838.39</td>
</tr>
<tr>
<td><strong>T (K)</strong></td>
<td>95</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td><strong>Crystal system, space group</strong></td>
<td>Monoclinic, C 2/c</td>
<td>Triclinic, P–1</td>
<td>Triclinic, P–1</td>
</tr>
<tr>
<td><strong>Unit cell dimensions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>a (Å)</strong></td>
<td>22.6910 (14)</td>
<td>8.7031 (1)</td>
<td>11.9157 (5)</td>
</tr>
<tr>
<td><strong>b (Å)</strong></td>
<td>7.0280 (3)</td>
<td>14.9274 (2)</td>
<td>13.0812 (6)</td>
</tr>
<tr>
<td><strong>c (Å)</strong></td>
<td>13.5206 (7)</td>
<td>15.1670 (2)</td>
<td>13.3817 (7)</td>
</tr>
<tr>
<td><strong>α (°)</strong></td>
<td>90</td>
<td>93.267 (1)</td>
<td>68.410 (5)</td>
</tr>
<tr>
<td><strong>β (°)</strong></td>
<td>100.504 (5)</td>
<td>74.65 (1)</td>
<td>74.07 (1)</td>
</tr>
<tr>
<td><strong>γ (°)</strong></td>
<td>109.84 (2)</td>
<td>164.1 (1)</td>
<td>167.1 (1)</td>
</tr>
<tr>
<td><strong>V (Å$^3$)</strong></td>
<td>2120.0 (2)</td>
<td>1904.09 (4)</td>
<td>1829.80 (16)</td>
</tr>
<tr>
<td><strong>Z</strong></td>
<td>8</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Goodness-of-fit on F[K]</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Final R indices [I&gt;2σ(I)]</strong></td>
<td>R$_1 = 0.0613$, wR$_2 = 0.1619$</td>
<td>R$_1 = 0.0310$, wR$_2 = 0.0819$</td>
<td>R$_1 = 0.0374$, wR$_2 = 0.0925$</td>
</tr>
<tr>
<td><strong>R indices (all data)</strong></td>
<td>R$_1 = 0.0667$, wR$_2 = 0.1669$</td>
<td>R$_1 = 0.0334$, wR$_2 = 0.0842$</td>
<td>R$_1 = 0.0457$, wR$_2 = 0.0980$</td>
</tr>
<tr>
<td><strong>Largest peak and hole/e Å$^{-3}$</strong></td>
<td>0.595/–0.396</td>
<td>0.482/–0.303</td>
<td>0.835/–0.656</td>
</tr>
</tbody>
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Table 2: Hydrogen bonds (Å) and angles (°) for H$_2$L-MeOH, 1 and 2.

<table>
<thead>
<tr>
<th>D-H···A</th>
<th>d(D-H)</th>
<th>d(H-A)</th>
<th>d(D···A)</th>
<th>&lt;(D-H-A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$L-MeOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1-H1N1···O1</td>
<td>0.89</td>
<td>1.79</td>
<td>2.682 (2)</td>
<td>174.3</td>
</tr>
<tr>
<td>N5-H1N5···N4$^a$</td>
<td>0.91</td>
<td>2.07</td>
<td>2.947 (2)</td>
<td>162.3</td>
</tr>
<tr>
<td>O1-H1···N3$^b$</td>
<td>0.84</td>
<td>1.97</td>
<td>2.795 (2)</td>
<td>166.2</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N53-H1N5···O1W</td>
<td>0.88</td>
<td>2.00</td>
<td>2.8660 (18)</td>
<td>166.9</td>
</tr>
<tr>
<td>O1W-H1O1···O1D</td>
<td>0.83</td>
<td>1.91</td>
<td>2.7377 (18)</td>
<td>173.7</td>
</tr>
<tr>
<td>O1W-H2O1···O2D</td>
<td>0.91</td>
<td>1.87</td>
<td>2.7651 (18)</td>
<td>167.1</td>
</tr>
<tr>
<td>2$^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N53-H1N3···N24$^a$</td>
<td>0.94</td>
<td>2.33</td>
<td>3.144 (2)</td>
<td>143.9</td>
</tr>
<tr>
<td>N53-H1N3···N14$^a$</td>
<td>0.94</td>
<td>2.69</td>
<td>3.388 (2)</td>
<td>131.6</td>
</tr>
<tr>
<td>N54-H1N4···N23$^b$</td>
<td>0.93</td>
<td>2.31</td>
<td>3.098 (2)</td>
<td>143.0</td>
</tr>
<tr>
<td>O1E-H1E···N13</td>
<td>0.84</td>
<td>2.12</td>
<td>2.939 (2)</td>
<td>164.1</td>
</tr>
</tbody>
</table>

Symmetry transformations used to generate equivalent atoms. $^a$: x, y, z; $^b$: x + 1, y, z; $^c$: x, y + 1, z; $^d$: x, −y + 1, z; $^e$: −x + 1, −y + 2, z; $^f$: −x + 1, −y + 1, −z + 1.

The copper atom is coordinated by two bidentate phen ligands and two monodentate anionic H$_2$L$^-$ ligands, thus forming [CuN$_5$]$_2$ chromophore with the respective Cu-N distances listed in Table 3. Due to Jahn-Teller effect, complex [Cu(phen)$_2$(H$_2$L)$_2$] of 2 shows elongated square-bipyramidal geometry, in which the axial positions are occupied by nitrogen atoms of two phen ligands (Figure 3(a)). Moreover, the formation of supramolecular dimers is observed in the crystal structure of 2. These supramolecular dimers are stabilized by hydrogen bonds between nitrogen atom of the tetrazole ring and protonated nitrogen of indole of the second complex with following donor-acceptor distances: d(N54···N23) = 3.098 (2) Å (Figure 3(b) and Table 2). Furthermore, the supramolecular dimer is additionally stabilized by π-π stacking interactions formed by neighboring 1,10-phenanthrolines and indoles with the shortest distance between benzene and indole centroids of 3.5806 (12) Å (Table 4 and Figure 3(b)). Lastly, a hydrogen bond is also present between nitrogen atom of the tetrazole unit and ethanol molecule with d(O1E···N33) = 2.939 (2) Å (Figure 3(b)).
Table 3: Selected interatomic parameters (Å, °) for coordination compounds 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>[Cu(phen)$_2$(HL)]ClO$_4$·H$_2$O·2DMF (1)</th>
<th>[Cu(phen)$_2$(HL)$_2$]·EtOH (2)</th>
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Figure 2: Continued.
compounds. In our case, this represents a measure of the ability of studied complexes to scavenge detrimental radicals present in the intracellular environment, such as the aforementioned OH radical.

The DPPH scavenging activity for 1 and 2 was measured by UV-Vis spectroscopy at 517 nm in triplicate and the values were averaged for each concentration (Figure 4). Conveniently, the studied complexes show no significant absorption in this region (Figures S10 and S11). Evidently, both prepared complexes 1 and 2 possess DPPH radical scavenging activity, which increases with the increasing concentration of the copper complex (Figure 4). For comparison, we also measured the DPPH radical scavenging activity of ascorbic acid, which is much larger than those of both our complexes. Compound 2 shows slightly higher activity than complex 1. As the composition of both complexes is very similar, a possible reason for activity discrepancy between complexes 1 and 2 might be caused by the difference in their coordination polyhedra. Different geometries produce different ligand fields, which in turn affect energetic levels and splitting of d-orbitals. IY—his consequently affects both the kinetic and thermodynamic behavior of the DPPH quenching reaction [22, 29, 30].

To elucidate possible reaction mechanism of the antioxidant activity of 1 and 2, we performed theoretical calculations at DFT level of theory for complexes [Cu(phen)$_2$(HL)]$^+$ of 1 and [Cu(phen)$_2$(HL)$_2$] of 2. Herein, ORCA 5.0 computation package [31, 32] was utilized together with ωB97M-D4 range-separated hybrid functional [33–36]. The triple-zeta bases sets def2-TZVP(-f) were used for all atoms [37] and the calculations were speeded up by using def2/J auxiliary basis [38] and RIJCOSX approximation [39–41]. As the experimental data were acquired with methanolic solutions, the geometry optimizations of the respective complexes were done with the conductor-like polarizable continuum model (C-PCM) using parameters for methanol solvent [42, 43]. The thermochemistry data were calculated as implemented in ORCA at 298.15K and Gibbs free energies were corrected by where the factor of 1.89 kcal/mol is due to the change in standard state from gas phase to solution phase [44].

First, the hydrogen atom transfer (HAT) mechanism was evaluated with the help of the following reactions:

$$[\text{Cu(phen)}_2(\text{HL})]^+ \rightarrow [\text{Cu(phen)}_2(\text{L})]^+ + \text{H}^-$$

($\Delta G = 87.13$ kcal/mol)
\[ \text{[Cu(phen)\textsubscript{2}(HL)\textsubscript{2}] \leftrightarrow [Cu(phen)\textsubscript{2}(HL) (L)] + H^+} \quad (\Delta_r G = 85.68 \text{ kcal/mol}) \]

Next, we considered single electron transfer (SET) resulting in the solvated electron release/absorption as written here:

\[ \text{[Cu(phen)\textsubscript{2}(HL)]^+ + CH\textsubscript{3}OH \leftrightarrow [Cu(phen)\textsubscript{2}(L)]^{2+} + CH\textsubscript{3}OH^-} \quad (\Delta_r G = 125.92 \text{ kcal/mol}) \]

\[ \text{[Cu(phen)\textsubscript{2}(HL)\textsubscript{2}]^+ + CH\textsubscript{3}OH \leftrightarrow [Cu(phen)\textsubscript{2}(HL)\textsubscript{2}]^+ + CH\textsubscript{3}OH^-} \quad (\Delta_r G = 144.18 \text{ kcal/mol}) \]

Figure 3: (a) ORTEP drawing of 50% probability with atom-numbering scheme for the asymmetric unit of 2. (b) The part of the crystal structure showing the formation of supramolecular dimers through the hydrogen bonds and π-π stacking, only the hydrogen atoms bonded to nitrogen and oxygen are shown.
Finally, the last evaluated mechanism is proton loss (PL), where the deprotonation of the nitrogen atom of the indole part of HL− was considered:

\[ \text{[Cu(phen)₂(HL)⁺] + CH₃OH} \rightleftharpoons \text{[Cu(phen)₂(L)⁺] + CH₃OH} \quad (\Delta_r G = 52.22 \text{kcal/mol}) \]

\[ \text{[Cu(phen)₂(HL)₂⁺] + CH₃OH} \rightleftharpoons \text{[Cu(phen)₂(HL)(L)⁺] + CH₃OH} \quad (\Delta_r G = 52.17 \text{kcal/mol}) \]

Evidently, the only spontaneous reaction (exergonic reactions) is attributed to SET in which the electron is donated to the complexes, which resulted in the reduction of Cu²⁺ to Cu¹. Moreover, the \( \Delta_r G = -87.26 \text{ kcal/mol} \) for \([\text{Cu(phen)₂(HL)}]⁺\) of 1 and the value of \( \Delta_r G = -81.58 \text{ kcal/mol} \) for \([\text{Cu(phen)₂(HL)₂⁺}]) \) of 2 are similar in agreement with the comparable radical scavenging activity of these compounds.

4. Conclusions

The impact of different copper salts on the preparation of metal complexes with 2-((H-tetrazol-5-yl)-1H-indole ligand (H₂L) was investigated. The single-crystal X-ray analysis confirmed formation of pentacoordinate \([\text{Cu(phen)₂(HL)}]\) ClO₄.2H₂O.2DMF (1) and hexacoordinate \([\text{Cu(phen)₂(HL)₂}]\)·EtOH (2) compounds. In both compounds, the anionic HL− ligand acts as a monodentate N-donor ligand attached to the central atom through the tetrazole unit. The investigation of the interaction of these complexes with DPPH radical in their methanolic solution revealed moderate radical scavenging activity. The subsequent theoretical DFT calculations proposed that the dominant mechanism is the single electron transfer to the studied complexes.

Data Availability

The data used to support the findings of this study are included within the article and the supplementary information file.

Conflicts of Interest

The authors declare that there are no conflicts of interest that could influence the work reported in this study.

Acknowledgments

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Supplementary Materials

Figures S1–S5: NMR spectra of H₂L. Figures S6–S9: FT-IR spectra of H₂L, phen, and 1 and 2. Figures S10 and S11: UV-VIS spectra of 1 and 2. XYZ coordinates of DFT-optimized molecular geometries. (Supplementary Materials)

References


