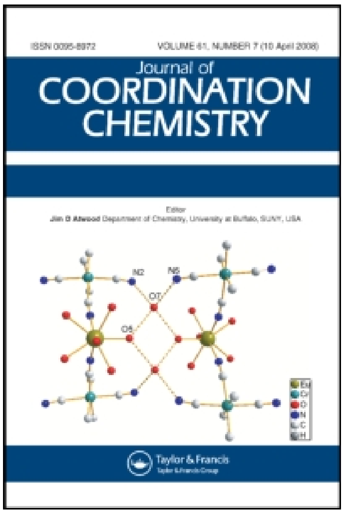

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Synthesis, characterization, and superoxide dismutase activity of copper(II) complexes with bidentate ligands

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Synthesis, characterization, and superoxide dismutase activity of copper(II) complexes with bidentate ligands

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Two new copper(II) complexes, $[Cu(BH)(bipy)](ClO_4)_2(H_2O)_2$ (**1**) and $[Cu(BH)_2(NO_3)_2]$ (**2**) (BH = benzoylhydrazine, bipy = 2,2′-bipyridine), have been synthesized and characterized. X-ray crystallographic analysis shows the copper to be four- and six-coordinate in the complexes. The magnetic and spectroscopic data indicate a distorted square-planar geometry for **1**, while **2** is assigned a distorted octahedral geometry. The electron paramagnetic resonance spectra indicate a $d_{x^2-y^2}$ ground state ($g_{\|} > g_{\perp} > 2.0023$). Both complexes display quasireversible redox waves due to Cu(II)/Cu(I) reduction. The superoxide dismutase-mimetic activity of the complexes was investigated by nitro blue tetrazolium chloride DMSO assay.

Keywords: Copper(II) complexes; Crystal structure; SOD-like activity

1. Introduction

Copper is essential for plants and human beings in the form of micronutrient. More copper is found in the brain [1] and heart than in any other tissue except liver, where it is stored as copper thionein. The high metabolic rate of heart and brain requires relatively large amounts of copper metalloenzymes including tyrosinase, cytochrome, etc. Whenever copper deficiency occurs in the body due to unbalanced diet, it leads to brain diseases, anemia, and heart disease (especially in infants). The biological role of copper [2, 3] has stimulated research in the inorganic chemistry of copper. Its importance is not only in the chemistry of copper proteins, for which Cu(I), Cu(II), and Cu(III) species are relevant, but also in systems where more than one type of copper is present. Copper(II) coordination complexes have biological activity of interest in pharmacology. The presence of excess copper can be as deleterious as an insufficient amount. Thus, accidental ingestion can lead to the impossibility of normal function of the natural biochemical mechanisms of detoxification. For example, Wilson's disease results from a genetically inherited metabolic defect in which copper cannot be tolerated even at normal levels [4].

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Bidentate ligands have been extensively used in coordination chemistry of copper(II) with the possibility of obtaining low-symmetry five- or six-coordinate complexes. Benzoylhydrazones are extensively used as an important class of nitrogen–oxygen donors, particularly for transition metal ions. Johnson *et al.* [5] found that benzoylhydrazone derivatives have strong insecticidal activity useful to control a variety of insects including mosquitoes (*Culex pipiens pallens*, *Culex pipiens molestus*, *Aedes aegypti*, *Aedes togoi*, *Anopheles sinensis*, etc.), house fly (*Musca domestica*), flesh flies, rice corn fly (Thiplaaino), rice midge (*Chironomus oryzae*), soyabean pod gall midge (*Asphondylia* sp.), oriental fruit fly (*Dacus dorsalis*), melon fly (*Dacus cucurbitae*), rice leaf miner (*Hydrella griscola*), rice whorl maggot (*Hydrella sasakii*), rice stem maggot (*Chlorops ozyzae*), stone leak leaf miner (*Liriomyza chinensis*), onion maggot (*Hylemya anhinga*), seedcorn maggot (*Hylemya platura*), etc.

As an extension of our work [6, 7], we now report the preparation, crystal structures, and superoxide dismutase (SOD) activities of two new copper(II) complexes synthesized in 1 : 1 and 1 : 2 ratios. The benzoylhydrazine is chosen because the structures of such complexes are few and they can form an abundance of hydrogen bonds and different geometry structures essential to mimic SOD activity. Our aim is to synthesize new complexes, modifying the coordination environment of SOD-mimetic activity for better understanding of the SOD enzyme.

2. Experimental

2.1. *Materials*

Copper(II) nitrate trihydrate, sodium azide, and ammonium thiocyanate were purchased from SD fine-Chemicals, India. All other chemicals were of synthetic grade and used as received.

2.2. *Synthesis*

The reported complexes were synthesized by the following procedures.

2.2.1. [Cu(BH)(bipy)]$(ClO_4)_2(H_2O)_2$ (1). A methanolic solution (10 mL) of BH (1 mmol, 0.136 g) was added to a solution of $Cu(NO_3)_2 \cdot 3H_2O$ (1.0 mmol, 0.242 g) followed by methanolic solution of bipy (1 mmol 0.156 g) in the same solvent (10 mL) and stirred for 30 min at room temperature (RT). To this reaction mixture $NaClO_4$ was added (2.0 mmol, 0.244 g) and further stirred for 20 min. After a few days products suitable for X-ray analysis were collected, washed, and then air dried. Blue crystals of the complexes were stored in a $CaCl_2$ dessicators. Anal. Calcd for $C_{15.75}H_{16}Cl_2CuN_4O_{11}$ (**1**) (%): C, 33.05; H, 2.79; N, 9.79. Found (%): C, 33.21; H, 2.70; N, 9.71. Fast atomic bombardment (FAB) mass (*m*/*z*) Calcd: 571.77. Found: 571.02.

2.2.2. Synthesis of [Cu(BH)$_2$(NO_3)$_2$] (2). To a MeOH solution (10 mL) of $Cu(NO_3)_2 \cdot 3H_2O)$ (1.0 mmol, 0.242 g) a MeOH solution (10 mL) of BH (2 mmol, 0.272 g) was added while stirring for 30 min at RT and stirred further for 30 min.

After completion of the reaction, blue crystals were collected, washed with methanol and diethyl ether, dried in air at RT, and stored in a $CaCl_2$ dessicator. Anal. Calcd for $C_{14}H_{16}CuN_6O_8$ (**2**) (%): C, 36.53; H, 3.47; N, 18.26. Found (%): C, 36.47; H, 3.33; N, 18.11. FAB mass (*m*/*z*) Calcd: 459.87. Found: 459.79.

2.3. *Physical measurements*

2.3.1. FAB mass spectra. FAB mass spectra were recorded on a JEOL SX 102/DA 6000 Mass Spectrometer using xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at RT with *m*-nitrobenzoyl alcohol as the matrix.

2.3.2. Magnetic measurements. RT magnetic susceptibilities were measured by Gouy balance using a mercury(II) tetrathiocyanato cobaltate(II) as calibrating agent ($\chi_g = 16.44 \times 10^{-6}$ c.g.s. units). Diamagnetic corrections were estimated from Pascal's tables.

2.3.3. Spectrometry. Ligand field spectra were recorded at 25°C on a Shimadzu UV-Vis recording Spectrophotometer UV-1601 in solution. Infrared (IR) spectra were recorded in KBr disks on a Perkin-Elmer 783 spectrophotometer. X-band electron paramagnetic resonance (EPR) spectra were recorded on a Varian E-line Century Series Spectrometer equipped with a dual cavity and operating at X-band with 100 kHz modulation frequency. TCNE was used as field marker.

2.3.4. Electrochemistry. Cyclic voltammetry (CV) was carried out with a BAS-100 Epsilon electrochemical analyzer having an electrochemical cell with a three-electrode system. Ag/AgCl was used as a reference electrode, glassy carbon as working electrode, and platinum wire as an auxiliary electrode. $NaClO_4$ ($0.1\,mol\,L^{-1}$) was used as supporting electrolyte and DMSO as solvent. All measurements were carried out at 298 K under a nitrogen atmosphere.

2.3.5. SOD activity. The *in vitro* SOD activity was measured using alkaline DMSO as a source of superoxide radical (O_2^-) and nitro blue tetrazolium chloride (NBT) as an O_2^- scavenger [8, 9]. In general, 400 µL sample to be assayed was added to a solution containing 2.1 mL of $0.2\,mol\,L^{-1}$ potassium phosphate buffer (pH 8.6) and 1 mL of $56\,\mu mol\,L^{-1}$ alkaline DMSO solution was added while stirring. The absorbance was then monitored at 540 nm against a sample prepared under similar conditions except that NaOH was absent in DMSO. A unit of SOD activity is the concentration of complex, which causes 50% inhibition of alkaline-DMSO-mediated reduction of NBT.

2.4. *Crystal structure determination*

Crystals suitable for X-ray analysis for $[Cu(BH)(bipy)](ClO_4)_2(H_2O)_2$ (**1**) and $[Cu(BH)_2(NO_3)_2]$ (**2**) were grown from slow evaporation of the reaction mixtures at

RT and mounted on a glass fiber for data collection. Crystal data were collected on an Enraf–Nonius MACH$_3$ diffractometer using graphite-monochromated Mo-Kα radiation ($\lambda = 0.71073$ Å). The crystal orientation, cell refinement, and intensity measurements were made using CAD-4PC performing ψ-scan measurements. The structures were solved by direct methods using SHELXS-97 [10] and refined by full-matrix least-square techniques against F^2 using SHELXL-97 [11]. All non-hydrogen atoms were refined anisotropically. All hydrogens were placed at calculated positions and were allowed to refine using a riding model.

3. Results and discussion

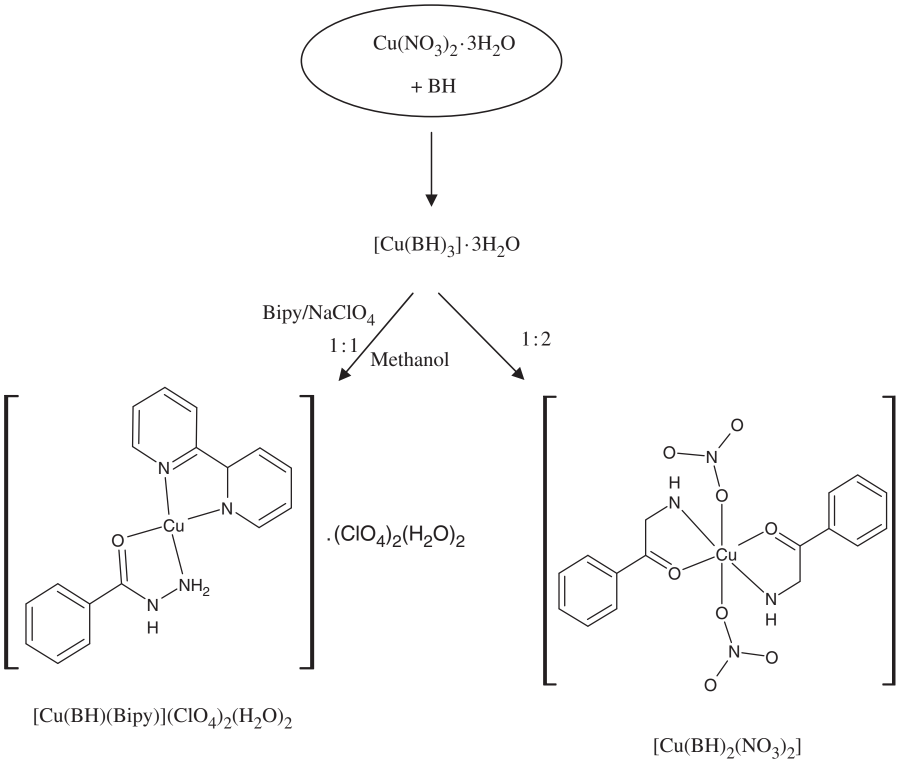
3.1. *Synthesis*

For the synthesis of [Cu(BH)(bipy)]$(ClO_4)_2(H_2O)_2$ (**1**), stoichiometric copper nitrate trihydrate and ligands were mixed. The product obtained immediately and the mixture was left overnight. Slow evaporation of the solvent gives the crystallized complex. $[Cu(BH)_2(NO_3)_2]$ was obtained by stirring methanolic solution of BH and $Cu(ClO_4)_2 \cdot 3H_2O$ in a 2 : 1 molar ratio at RT. Ring closure takes place without elimination of any molecule resulting in the formation of the complex. The green solution obtained after the reaction was filtered and upon slow evaporation of the solvent, the complex crystallized after 1 week. The general synthetic route of complexes is shown in scheme 1.

3.2. *Crystal structures*

Figures 1 and 2 display the structure of **1** and **2** with their labeling scheme. The crystal structure refinement data of **1** and **2** are given in table 1 and selected interatomic distances and angles are listed in tables 2 and 3, respectively. In **2** the geometry of copper(II) can best be described as very slightly distorted square planar. Cu(II) is bonded with two pyridyl nitrogens, one carbonyl oxygen, and one hydrazinic nitrogen. Cu(1)–N(2) is 2.004(10) Å with hydrazinic nitrogen and Cu(1)–N(4) is 2.000(12) Å for 2,2′-bipyridine; Cu(1)–O(1) is 1.941(14) Å with carbonyl oxygen and Cu(1)–N(3) is 1.943(16) Å with 2,2′-bipyridine. The bite angles for $CuC_1O_1N_2$ and CuC_2N_2 chelate rings are 84.06 and 82.33, respectively. The geometry around the Cu(II) square plane is distorted square planar, evident from the value *trans* angles N(2)–Cu(1)–N(4) 177.7(5)° and O(1)–Cu(1)–N(3) 176.4(6)°. The *cis* angles O(1)–Cu(1)–N(2) 84.1(5)°, N(2)–Cu(1)–N(3) 99.5(6)°, N(3)–Cu(1)–N(4) 82.4(6)°, and N(4)–Cu(1)–O(1) 94.2(5)° illustrate the slight distortion from square-planar geometry. The ligand to metal bond distances are in the range 1.941(14)–2.004(10) Å, comparable to the values found in similar Cu(II) complexes [12, 13].

In **2** the coordination geometry about copper can be described as an elongated and strongly distorted octahedron, where O333–Cu–O333 occupy axial positions (figure 3). The Cu–O333 distance is large (2.568 Å), so that O333 can be considered as semi-coordinated [14, 15] and the coordination geometry about the copper can be viewed as 4 + 2. In the monoclinic mononuclear $[Cu(BH)_2(NO_3)_2]$, each Cu(II) is located at the inversion center. The six-membered chelate ring is coplanar but the two five-membered



(1) **(2)**

Scheme 1. Synthesis of complexes **1** and **2**.

chelate rings are not parallel to each other, separated by 3.131 Å. Two ligands coordinate to Cu(II) with carbonyl oxygen and hydrazinic nitrogen *cis*. Cu(II) is bonded with two carbonyl oxygens, two amino nitrogens, and two nitro oxygens with Cu1–O1 = 1.9424(13) Å, Cu–N1 = 1.9729(16) Å, and Cu–O333 = 2.568 Å, respectively (figure 2). Thus the ligand is neutral bidentate chelating (scheme 1). The Cu–N bond length in **2** agrees well with those found for **1**. The Cu–O bond length is comparatively shorter than the sum of van der Waal's radii of oxygen and copper (1.50 + 1.40 = 2.90 Å), indicating strong Cu–O bonding [16].

The crystal packing reveals existence of $\pi \cdots \pi$ and C–H$\cdots$O intermolecular interactions, stabilized by a complicated hydrogen-bonding network.

In the lattice structure, **1** is interwoven through the intermolecular and intramolecular hydrogen-bonding interactions (table 4). Perchlorate forms relatively weaker hydrogen bonds. The conventional N(2)–H(2)$\cdots$O(666) (2.988(0.017) Å) hydrogen bonds along with unconventional weaker C–H$\cdots$O (3.826(0.037) Å) intermolecular hydrogen bonds and $\pi \cdots \pi$ intermolecular interactions form a zig-zag network. The benzene ring of BH and the pyridine ring of bipy form continuous $\pi \cdots \pi$ stacking. The intermolecular stacking distances between centroids cg_1 of benzene ring and cg_2 of bipy is 4.148 Å. The distances between cg_3 of bipy and cg_4 of benzene is 3.636 Å. Complex **2** is also interwoven through the intermolecular and intramolecular hydrogen-bonding

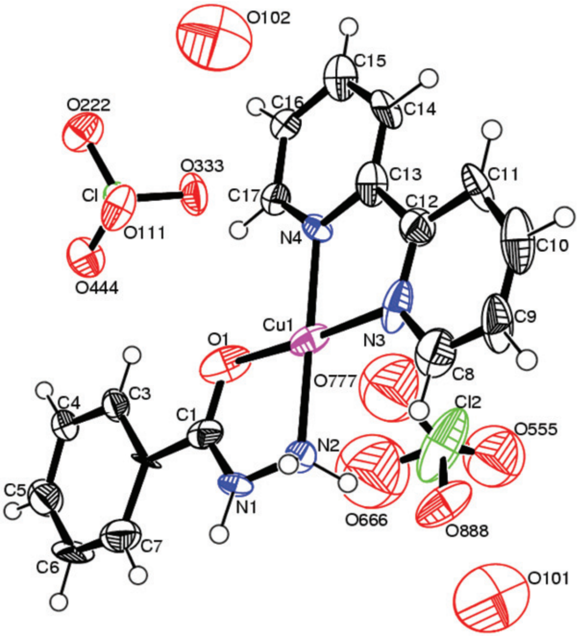


Figure 1. ORTEP view of $[Cu(BH)(bipy)](ClO_4)_2(H_2O)_2$ (**1**).

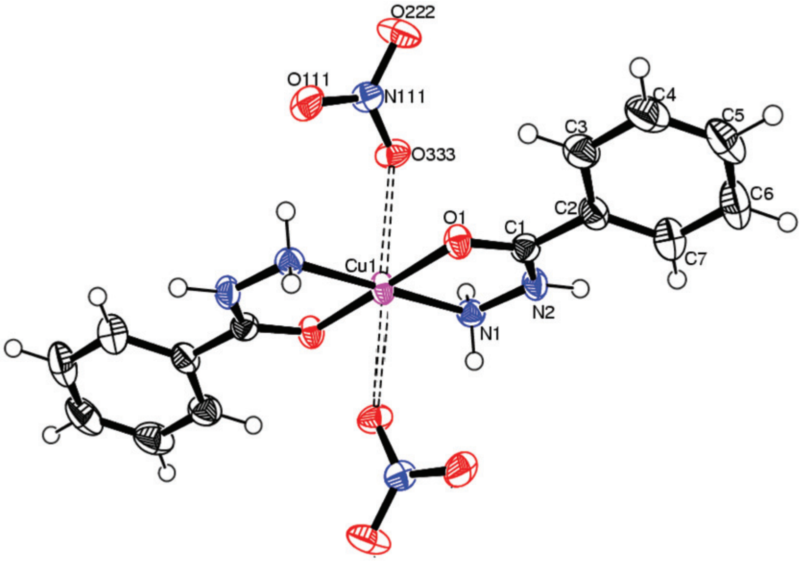


Figure 2. ORTEP view of $[Cu(BH)_2(NO_3)_2]$ (**2**).

interactions (table 4). The conventional strong $N5–H5\cdots O666$ (2.995(0.021) Å) hydrogen bonds along with unconventional weaker $C–H\cdots O$ (3.826(0.037) Å) intermolecular hydrogen bonds are also present. All the coppers form square boxes when viewed along the *a*-axis (figure 4) with Cu–Cu distance of 6.962 Å.

Table 1. Crystal structure data refinement parameter for **1** and **2**.

Identification code	**1**	**2**
Empirical formula	$C_{15.75}\,H_{16}Cl_2CuN_4O_{11}$	$C_{14}H_{16}CuN_6O_8$
Formula weight	571.77	459.87
Temperature (K)	120(2)	120(2)
Wavelength (λ) (Å)	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic
Space group	*C2/c*	*P2₁/c*
Unit cell dimensions (Å, °)		
a	24.574(3)	10.0881(10)
b	13.8517(12)	10.1437(10)
c	13.5334(18)	9.5389(9)
α	90	90
β	97.515(13)	103.357(10)
γ	90	90
Volume (A^3), Z	4567.1(9), 8	949.72(16), 2
Calculated density ($Mg\,m^{-3}$)	1.663	1.608
Absorption coefficient (mm^{-1})	1.255	1.206
$F(000)$	2316	470
Crystal size	$0.23 \times 0.16 \times 0.12$	$0.33 \times 0.28 \times 0.23$
Theta range for data collection (°)	2.94–25.00	3.32–25.00
Limiting indices	$-29 \le h \le 29$, $-16 \le k \le 16$, $-16 \le l \le 13$	$-11 \le h \le 11$, $-12 \le k \le 12$, $-10 \le l \le 11$
Reflections collected/unique	13,409/4003 [R(int) = 0.0361]	7474/1663 [R(int) = 0.0205]
Completeness to $\theta = 25.0$ (%)	99.5	99.8
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	0.8640 and 0.7612	0.7688 and 0.6916
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	4003/192/301	1663/0/145
Goodness-of-fit on F^2	1.165	1.073
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.1713$, $wR_2 = 0.3964$	$R_1 = 0.0231$, $wR_2 = 0.0655$
R indices (all data)	$R_1 = 0.1893$, $wR_2 = 0.4037$	$R_1 = 0.0295$, $wR_2 = 0.0668$
Largest difference peak and hole (e $Å^{-3}$)	2.669 and −1.660	0.250 and −0.275

3.3. *EPR spectra*

EPR spectra of the complexes in the polycrystalline state at 298 K and in solution at 77 K were recorded in the X-band region using 100 KHz field modulation; g factors were noted relative to the standard marker TCNE ($g = 2.0277$). EPR spectral assignment of the copper(II) complexes along with the spin Hamiltonian and orbital reduction parameters are given in table 5. Some representative spectra are shown in Supplementary material. The EPR spectra of **1** and **2** in the polycrystalline state at 298 K show only one broad signal. Such an isotropic spectrum arises from extensive exchange coupling through misalignment of the local molecular axes between different molecules in the unit cell and enhanced spin lattice relaxation, giving no information on the electronic ground state of the Cu(II).

The solution EPR spectra of the complexes were recorded in DMSO at 77 K. Frozen solution EPR spectra of the complexes reveal axial features ($g_{\|} > g_{\perp} > 2.0023$) and suggest a $d_{x^2-y^2}$ ground state, characteristic of axial symmetry. The EPR parameters $g_{\|}$, $g_{\perp}$, $A_{\|}$ and the energies of the d–d transition were used to evaluate the bonding parameters α^2, β^2, and γ^2, which may be regarded as measures of the covalency of the in-plane π bonds. The values of G are 4.8 and 3.1 for **1** and **2**, indicating out-of-exchange and in-plane exchange interaction in the solid state.

Table 2. Selected bond distances (Å) and angles (°) of **1**.

Cu(1)–N(3)	1.948(16)	Cu(1)–N(2)	2.004(10)
Cu(1)–O(1)	1.941(14)	Cu(1)–N(4)	2.000(12)
N(3)–Cu(1)–O(1)	176.4(6)	C(4)–C(3)–C(2)	119.2(15)
N(3)–Cu(1)–N(2)	99.5(6)	C(3)–C(4)–C(5)	119.0(17)
O(1)–Cu(1)–N(2)	84.1(5)	C(6)–C(5)–C(4)	121.5(18)
N(3)–Cu(1)–N(4)	82.4(6)	C(7)–C(6)–C(5)	121.1(16)
O(1)–Cu(1)–N(4)	94.2(5)	C(6)–C(7)–C(2)	119.8(17)
N(2)–Cu(1)–N(4)	177.7(5)	C(9)–C(8)–N(3)	124(2)
N(1)–N(2)–Cu(1)	104.5(8)	C(8)–C(9)–C(10)	116.6(18)
C(12)–N(3)–Cu(1)	113.9(11)	C(9)–C(10)–C(11)	124.9(18)
C(8)–N(3)–Cu(1)	126.8(15)	C(10)–C(11)–C(12)	114.9(18)
C(17)–N(4)–Cu(1)	125.4(10)	N(3)–C(12)–C(11)	120.4(16)
C(13)–N(4)–Cu(1)	114.0(11)	N(3)–C(12)–C(13)	116.0(16)
C(1)–O(1)–Cu(1)	112.4(11)	C(11)–C(12)–C(13)	123.4(16)
O(1)–C(1)–N(1)	121.7(17)	N(4)–C(13)–C(14)	120.8(14)
O(1)–C(1)–C(2)	119.0(14)	N(4)–C(13)–C(12)	113.7(16)
N(1)–C(1)–C(2)	119.3(15)	C(14)–C(13)–C(12)	125.4(15)
C(7)–C(2)–C(3)	119.2(15)	C(13)–C(14)–C(15)	120.5(15)
C(7)–C(2)–C(1)	124.1(15)	C(16)–C(15)–C(14)	117.1(17)
C(3)–C(2)–C(1)	116.6(12)	C(15)–C(16)–C(17)	119.0(15)
		N(4)–C(17)–C(16)	121.8(15)

Table 3. Selected bond distances (Å) and angles (°) of **2**.

Cu(1)–O(1)	1.9424(13)	Cu(1)–N(1)#1	1.9729(16)
Cu(1)–O(1)#1	1.9424(13)	Cu(1)–N(1)	1.9729(16)
O(1)–C(1)	1.262(2)	N(1)–N(2)	1.413(2)
O(1)–Cu(1)–O(1)#1	180.00(2)	C(1)–O(1)–Cu(1)	112.15(11)
O(1)–Cu(1)–N(1)#1	96.35(5)	N(2)–N(1)–Cu(1)	106.71(11)
O(1)#1–Cu(1)–N(1)#1	83.65(5)	Cu(1)–N(1)–H(1BN)	110.9(14)
O(1)–Cu(1)–N(1)	83.65(5)	Cu(1)–N(1)–H(1AN)	109.2(13)
O(1)#1–Cu(1)–N(1)	96.35(5)	O(222)–N(111)–O(333)	120.98(15)
N(1)#1–Cu(1)–N(1)	180.00(2)	O(222)–N(111)–O(111)	118.83(15)
O(1)–Cu(1)–O(1)#1	180.00(2)	O(333)–N(111)–O(111)	120.19(15)

Symmetry transformations used to generate equivalent atoms: $1 - x + 1, -y, -z + 1$.

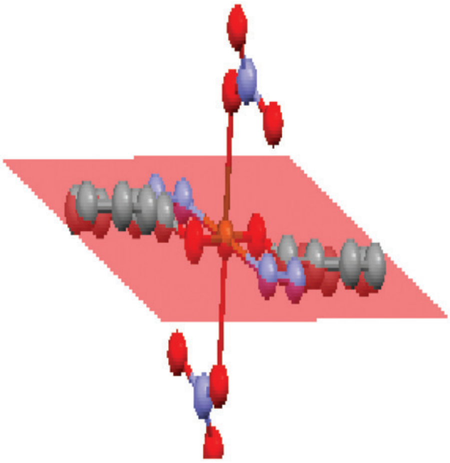


Figure 3. Defined plane and mode of coordination in **2**.

Table 4. Hydrogen-bonding interactions (Å and °).

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	∠D–H···A (°)	Symmetry
Intramolecular					
1					
C7–H7···N1 (0)	0.950(0.021)	2.676(0.015)	2.941(0.025)	96.59(1.27)	x, y, z
C8–H8···N2 (0)	0.950(0.022)	2.697(0.012)	3.222(0.025)	115.52(1.38)	x, y, z
C17–H17···O1 (0)	0.950(0.016)	2.488(0.013)	3.026(0.021)	115.83(1.04)	x, y, z
C3–H3···O1 (0)	0.950(0.016)	2.453(0.013)	2.772(0.021)	99.41(1.05)	x, y, z
2					x, y, z
C3–H3···O1 (0)	0.950(0.002)	2.494(0.001)	2.790(0.002)	98.00(0.13)	x, y, z
C7–H7···N2 (0)	0.950(0.002)	2.643(0.002)	2.904(0.003)	96.24(0.14)	x, y, z
Intermolecular					
1					
N2–H2A···Cl2 (0)	0.920(0.013)	2.942(0.008)	3.542(0.017)	124.19(0.92)	x, y, z
N2–H2A···O888 (0)	0.920(0.013)	2.157(0.015)	2.995(0.021)	151.09(0.98)	x, y, z
N2–H2A···O777 (0)	0.920(0.013)	2.783(0.030)	3.049(0.034)	97.92(1.02)	x, y, z
C3–H3···O111 (0)	0.950(0.016)	2.666(0.012)	3.558(0.020)	156.74(1.03)	x, y, z
C3–H3···O444 (0)	0.950(0.016)	2.985(0.014)	3.535(0.022)	118.27(1.03)	x, y, z
C4–H4···O444 (0)	0.950(0.017)	2.696(0.013)	3.393(0.022)	130.75(1.05)	x, y, z
C17–H17···O111 (0)	0.950(0.016)	2.460(0.011)	3.207(0.019)	135.45(1.00)	x, y, z
C7–H7···O101 (1)	0.950(0.021)	2.947(0.031)	3.826(0.037)	154.36(1.37)	$x, -y+1, +z+1/2$
N1–H1···O101 (1)	0.880(0.015)	2.039(0.032)	2.812(0.035)	145.94(1.30)	$x, -y+1, +z+1/2$
C6–H6···O333 (2)	0.950(0.016)	2.816(0.012)	3.252(0.021)	109.00(1.12)	$-x+1/2, +y+1/2, -z+1/2$
N1–H1···O102 (2)	0.880(0.015)	2.867(0.034)	3.423(0.037)	122.63(1.20)	$-x+1/2, +y+1/2, -z+1/2$
C5–H5···O777 (2)	0.950(0.020)	2.559(0.030)	3.295(.037)	134.52(1.38)	$-x+1/2, +y+1/2, -z+1/2$
C8–H8···O888 (3)	0.950(0.022)	2.557(0.016)	3.462(0.027)	159.46(1.36)	$-x, +y, -z+1/2$
N2–H2A···O888 (3)	0.920(0.013)	2.393(0.013)	2.988(0.017)	122.31(0.85)	$-x, +y, -z+1/2$
N2–H2B···O222 (4)	0.920(0.015)	2.186(0.014)	3.013(0.020)	149.15(0.93)	$-x+1/2, -y+1/2, -z+1$
C8–H8···O222 (4)	0.950(0.022)	2.946(0.013)	3.345(0.024)	106.72(1.23)	$-x+1/2, -y+1/2, -z+1$
C10–H10···O444 (5)	0.950(0.023)	2.822(0.013)	3.402(0.025)	120.30(1.32)	$x-1/2, +y-1/2, +z$
C9–H9···O444 (5)	0.950(0.020)	2.558(0.013)	3.250(0.024)	129.82(1.24)	$x-1/2, +y-1/2, +z$
C10–H10···O222 (5)	0.950(0.023)	2.635(0.014)	3.554(0.027)	162.97(1.40)	$x-1/2, +y-1/2, +z$
C15–H15···O666 (6)	0.950(0.019)	2.278(0.034)	3.062(0.039)	139.38(1.42)	$-x+1/2, +y-1/2, -z+1/2$
C11–H11···O333 (6)	0.950(0.018)	2.732(0.013)	3.458(0.023)	133.74(1.12)	$-x+1/2, +y-1/2, -z+1/2$
Intramolecular					
2					
C4–H4···O222 (1)	0.950(0.002)	2.586(0.001)	3.374(0.002)	140.56(0.14)	$-x+2, -y, -z+2$
C6–H6···O222 (2)	0.950(0.002)	2.740(0.002)	3.643(0.003)	158.86(0.14)	$-x+2, +y+1/2, -z+1/2+1$
C5–H5···O111 (2)	0.950(0.002)	2.673(0.002)	3.428(0.003)	136.75(0.14)	$-x+2, +y+1/2, -z+1/2+1$
C5–H5···O333 (3)	0.950(0.002)	2.826(0.001)	3.674(0.003)	149.22(0.14)	$x+1, +y, +z$
N1–H1AN···N111 (4)	0.855(0.020)	2.856(0.020)	3.447(0.002)	127.86(1.69)	$-x+1, -y, -z+1$
N1–H1AN···O111 (4)	0.855(0.020)	2.047(0.021)	2.809(0.002)	148.13(1.94)	$-x+1, -y, -z+1$
N1–H1BN···N111 (5)	0.887(0.022)	2.876(0.022)	3.693(0.002)	153.73(1.73)	$-x+1, +y+1/2, -z+1/2+1$
N1–H1BN···O111 (5)	0.887(0.022)	1.974(0.021)	2.816(0.002)	158.01(1.87)	$-x+1, +y+1/2, -z+1/2+1$
N2–H2N···O222 (6)	0.906(0.021)	1.936(0.020)	2.774(0.002)	153.08(1.86)	$x, -y+1/2, +z-1/2$
N2–H2N···O333 (6)	0.906(0.021)	2.744(0.018)	3.203(0.002)	112.63(1.46)	$x, -y+1/2, +z-1/2$
N1–H1AN···O333 (6)	0.855(0.020)	2.615(0.020)	3.130(0.002)	119.88(1.61)	$x, -y+1/2, +z-1/2$
N2–H2N···O111 (6)	0.906(0.021)	2.682(0.020)	3.386(0.002)	135.26(1.62)	$x, -y+1/2, +z-1/2$
N1–H13N···O333 (6)	0.857(0.020)	2.918(0.020)	3.127(0.002)	85.27(1.33)	$x, -y+1/2, +z-1/2$

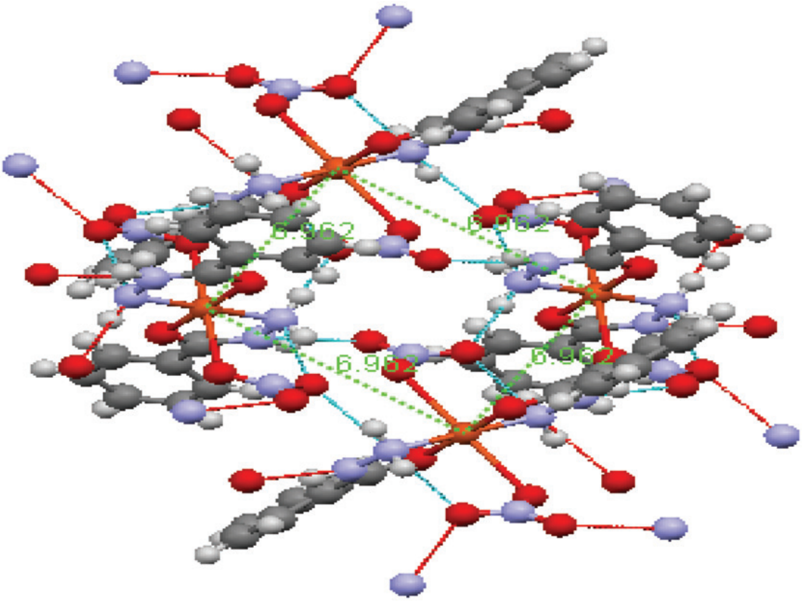


Figure 4. Unit cell packing diagram of **2** at *a*-axis.

Table 5. The EPR spectral parameters of the copper(II) complexes.

	1	**2**
Polycrystalline state (RT)		
g_{ave}	2.235	–
g_3	2.0465	–
g_{iso}	–	2.184
Frozen solution (LNT)		
$g_{\parallel}$	2.292	2.231
$g_{\perp}$	2.059	2.0764
$A_{\parallel}(G)$	150	160
G	4.8	3.1
α^2	0.741	0.721
β^2	1.036	1.158
γ^2	0.950	1.188
$K_{\parallel}$	0.791	0.675
$K_{\perp}$	0.704	0.757
f (cm)	160	150

From low-temperature EPR spectra of the complexes, various bonding parameters such as in-plane σ-bonding and in-plane π-bonding were evaluated [17, 18].

$$\alpha^2 = (A_{\parallel}/0.036) + (g_{\parallel} - 2.0023) + 3/7(g_{\perp} - 2.0023) + 0.04$$

The orbital reduction factors K and $K_{\perp}$ were estimated from the expressions [19]

$$K_{\parallel}^2 = (g_{\parallel} - 2.0023)E_{\mathrm{d-d}}/8\lambda_0$$

$$K_{\perp}^2 = (g_{\perp} - 2.0023)E_{\mathrm{d-d}}/2\lambda_0$$

where $K_{\|} = \alpha^2\beta^2$, $K_{\perp} = \alpha^2\gamma^2$, and λ_0 represents the one-electron spin-orbit coupling constant for the free ion, equal to $-828\,\mathrm{cm}^{-1}$. Information about the bonding in copper(II) complexes can be derived from the magnitude of $K_{\|}$ and $K_{\perp}$. For pure σ-bonding $K_{\|} \approx K_{\perp} \approx 0.77$, whereas $K_{\|} < K_{\perp}$ implies considerable in-plane bonding, while for out-of-plane bonding $K_{\|} > K_{\perp}$. $K_{\|} > K_{\perp}$ for **1** indicates the presence of out-of-plane bonding, whereas $K_{\|} < K_{\perp}$ for **2** indicates the presence of in-plane bonding. The values of α^2, β^2, and γ^2 of the complexes are consistent with both strong in-plane σ-bonding and in-plane π-bonding. The computed values of α^2 and β^2 (table 5) are compared with other copper(II) complexes [20]. Therefore, present complexes may be regarded as ionic complexes. A factor contributing to an increase of $g_{\|}$ is the distortion from square-planar geometry to a rhomboid (diamond) shape [21]. In contrast, low $g_{\|}$ values are common for six-coordinate copper(II) complexes [22, 23]. The degree of geometrical distortion has been approached by a parameter $f(g_{\|}/A_{\|}$ (in cm^{-1}) with values less than $140\,\mathrm{cm}^{-1}$ associated with square-planar structures, whereas higher values indicate tetrahedral distortion [24, 25]; for **1** the value of f is 160, in agreement with significant deviation from planarity. Together, with the high $g_{\|}$ value suggests weak fields with some tetrahedral distortion [26, 27], consistent with results from electronic spectroscopy. The relatively lower f value (150) for **2** indicates a moderate distortion from planarity, in agreement with results from electronic spectroscopy. The contribution of the 4s orbital to σ-bonding between metal and ligand donors is predominant in complexes having α values greater than 0.70 [28]. For these complexes, the values are nearly the value mentioned previously, which supports a ground term with purely 3d character.

3.4. *Electronic spectroscopy*

Electronic spectral data for complexes in DMSO ($3 \times 10^{-3}\,\mathrm{mol\,L^{-1}}$) are in good agreement with their geometries. Electronic spectra exhibit two absorptions. The first absorption (due to d–d transition) at 780 nm for **1** and 700 nm for **2** is assigned to $^2B_{1g} \rightarrow {}^2B_{2g}$, whereas the second band at 400 nm is assigned to a ligand to metal charge transfer [29]. According to Soptrajanov *et al.* [30] coordinated water should exhibit frequencies at 825, 575, and $500\,\mathrm{cm}^{-1}$. The absence of spectral band in these regions in the spectra of **1** indicates that water in these complexes is not coordinated but lattice water. Moreover, the solution spectra of the complexes shows bands at 355 and 305 nm assigned to the $n \rightarrow \pi^*$ transition, which may be due to distortion from square planar to tetrahedral. These absorption bands correspond to a distorted square-planar environment around copper(II) [31, 32], consistent with the geometry observed for structurally characterized copper(II) complexes [12].

3.5. *IR spectra*

For **1** and **2**, the bonding of donor to metal is evident from IR spectra, where the single strong C=N stretch is centered at 1591, 1596, 1598, and $1601\,\mathrm{cm}^{-1}$. The bands at 3114, 3183, 3196, and $3198\,\mathrm{cm}^{-1}$ for **1** and **2** are assigned to ν(NH) in benzoylhydrazone.

Coordination of metal is confirmed by bands at 578 and $525\,\mathrm{cm}^{-1}$ for ν(M–N) and 421 and $424\,\mathrm{cm}^{-1}$ for ν(M–O) for **1** and **2**. In both complexes a strong band at $266–280\,\mathrm{cm}^{-1}$ is consistent with ν(Cu–N) of pyridine as suggested by Clark and Williams [33]. The band at $268\,\mathrm{cm}^{-1}$ can be assigned to ν(Cu–ONO$_2$), consistent with

Table 6. CV data for 1 mmol L^{-1} solution of the Cu(II) complexes in DMSO containing 0.1 mol L^{-1} $NaClO_4$ as supporting electrolytes.

Scan rate (mV s^{-1})	E_{pc} (mV)	I_{pc} (μA)	E_{pa} (mV)	I_{pa} (μA)	ΔE_p (mV)	$E^{\circ\prime}$ (mV)	I_{pa}/I_{pc} (μA)
1							
100	−1091	1.826	256	3.838	−1347	−417	2.1
200	−1106	1.992	271	3.984	−1377	−417	2.0
300	−1131	2.158	295	4.152	−1426	−418	1.9
2							
100	35	1.950	315	4.089	280	175	2.0
200	32	2.212	320	4.433	288	176	2.0
300	29	2.450	325	4.924	296	177	2.1

$\Delta E_p = E_{pa} - E_{pc}$; $E^{\circ} = E_{pa} + E_{pc}/2$.

bands at 253–280 cm^{-1} reported earlier for Cu–ONO_2 [34]. In **1**, the presence of a band at ~1100 cm^{-1} and at ~625 cm^{-1} indicates that the T_d symmetry of ClO_4^- is maintained [35–37].

3.6. *Cyclic voltammetry*

The electrochemical properties of the complexes have been studied by CV in degassed DMSO (2×10^{-3} mol L^{-1}, Supplementary material). The data are given in table 6. The CV of **2** exhibits quasireversible Cu(II)/Cu(I) redox couple at $E_{1/2} = 114$ mV (*vs*. SCE), which indicates only one copper in the complex. The $\Delta E_p = 307$ at 100 V s^{-1} [38] indicates quasireversible behavior. Reduction peak (B) around −0.44 V (*vs*. SCE) was observed toward cathodic potential with its anodic peak (D) at about 0.85 V, attributed to the reduction of Cu(II) to Cu(I). The voltammogram is clearly quasireversible probably because after the reduction of Cu(II), the Cu(I) species thus formed is unstable. The negative value indicates a strong stabilization of copper(II) through the formation of complexes. The oxidation peak (E) at about 0.25 V and associated reduction peak (A) in the region 0.82 V at a scan rate of 100 V s^{-1} is assigned to the Cu(II)/Cu(I) redox couple. In the cathodic region, an irreversible peak at B is observed corresponding to the reduction of Cu(I) to Cu(0) [39, 40]. During the reverse scan, the oxidation peak Cu(I)/Cu(II) skips to the Cu(III) state, confirmed by the ratio of anodic to cathodic current value of the Cu(II)/Cu(III) couple ($I_{pc}/I_{pa} \approx 2$). The negative value indicates strong stabilization of copper(II) through the formation of the complex. Extra peaks beyond the first reduction may result from the electrochemical behavior (redox) of ligand on the electrode surface. The difference of Cu(II)/Cu(I) redox potentials between **1** and **2** demonstrates that coordination around Cu(II) of the two complexes is different and affords an explanation for the difference in SOD activity.

3.7. *SOD activity*

The SOD-like activities of the two complexes were investigated by NBT assay [41–43] and catalytic activity toward the dismutation of superoxide anion was measured. The macrocycle with two donor-set arms provides a stable and flexible environment similar

Table 7. IC_{50} values and kinetic constant of **1** and **2**.

Complex	IC_{50} (μmol)	k_{MccF} $((mol\,L^{-1})^{-1}\,s^{-1})^{b} \times 10^{4}$
1	42	2.26
2	48	1.98

k_{McCF} were calculated by $K = k_{NBT} \times [NBT]/IC_{50}$, k_{NBT} (pH 7.8) $= 5.94 \times 10^{4}$ $(mol\,L^{-1})^{-1}\,s^{-1}$ [48, 49].

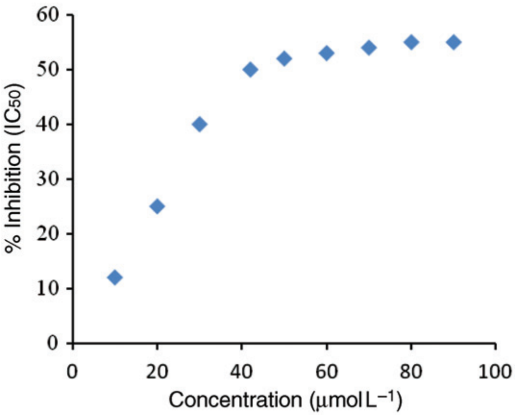


Figure 5. SOD activity of **1**.

to that in the active site of the native enzyme, ensuring the existence of the complexes. The chromophore concentration required to yield 50% inhibition of the reduction of NBT (IC_{50}) was determined by following the literature method [44]. The IC_{50} value and first-order rate constant for NBT assay for both complexes were also evaluated and presented in table 7. The IC_{50} values for **1** and **2** are 42 and 48 μmol L^{-1}, respectively (figure 5), among the most active model compounds but somewhat less active than the native enzyme [45–52]. The good activities of both complexes may be attributed to the flexible macrocyclic ligand [53].

3.8. *Biological activity*

The biological and medicinal potencies of coordination compounds have been established by antibacterial activities [54]. The synthesized complexes were screened for their antibacterial activity [55] against *Escherichia coli* at 10, 15, 20 mmol concentration. The zone-of-inhibitions of the antimicrobial activity are provided in Supplementary material. The complexes show weak activity against *E. coli*.

4. Conclusion

We have synthesized two complexes $[Cu(BH)(bipy)](ClO_4)_2(H_2O)_2$ and $[Cu(BH)_2(NO_3)_2]$ of benzoylhydrazine, which have the ability to effectively scavenge the superoxide ion (O_2^-). The complexes have strong and weak hydrogen-bonding interactions. Complex **1** exhibits weak $\pi \cdots \pi$ interactions which stabilize the molecule in solid state.

Supplementary material

CCDC 772113 and 772112 contain the supplementary crystallographic data for $[Cu(BH)(bipy)](ClO_4)_2(H_2O)_2$ (**1**) and $[Cu(BH)_2(NO_3)_2]$ (**2**) have been synthesized with benzoylhydrazine. These data can be obtained free of charge *via* http://www.ccdc.cam.3ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax (+44) 1223-336-033; or E-mail: deposit@ccdc.cam.ac.uk.

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