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Original article

Copper (II) complexes of the anti-inflammatory drug naproxen and 3-pyridylmethanol as auxiliary ligand. Characterization, superoxide dismutase and catecholase – mimetic activities

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ABSTRACT

The synthesis and spectral characterization of binary copper (II) complex of the non-steroidal anti-inflammatory drug naproxen (Nap) with formula $[Cu_2(Nap)_4]_n$ (**1**) and its ternary complex with 3-pyridylmethanol (3-pym) of formula $[Cu(Nap)_2(3\text{-pym})_2]_n$ (**2**) were investigated. Complex **1** is polymeric consisting of units of the known paddle-wheel dicopper (II) tetracarboxylates of four naproxenate ions bridging the two copper atoms. The units are axially connected through the neighboring carboxylate oxygen atoms. The X-ray molecular structure measurements of complex **2** showed that it is polymeric consisting of mononuclear units having trans–$CuN_2O_2 + O_2$ chromophores which are bridged by 3-pyridylmethanol ligands through their methanolic oxygen atoms. The measured superoxide dismutase (SOD) mimetic activities of the complexes indicated that complexes **1** and **2** are excellent SOD mimics with an IC_{50} of 0.30 μM for complex **1** and 0.39 μM for complex **2**. The catecholase activities of the complexes toward the aerobic oxidation of 3,5-di-tert-butylcatechol (DTBC) to 3,5-di-tert-butylquinone (DTBQ) showed that both complexes have moderate catalytic oxidase activities.

1. Introduction

Copper ions, as centers of active site of various metalloproteins, play a vital role in a number of widely differing biological processes like electron transfer, oxidation and dioxygen transport [1,2]. Small molecular weight copper complexes are studied as structural and functional models of active centers of copper containing enzymes [2–11]. Copper (II) complexes with non-steroidal anti-inflammatory drugs have been widely studied since they are found to be more active and desirable drugs than their parent drugs themselves [12,13]. In addition, binary and ternary copper (II) complexes of these drugs have been studied as models for copper containing enzymes such as Cu,Zn-superoxide dismutase (SOD) [9,12–17] and catechol oxidase [18,19]. SOD enzyme catalyzes the disproportion of the toxic superoxide anion $O^{\bullet}_2^-$ and protects the living cell against various pathological conditions involving cardiovascular diseases, cancer, inflammation, diabetes, other diseases and aging [20]. The clinical use of this SOD has many shortcomings, including its short life-time, high cost and low membrane permeability. So considerable efforts were made in order to obtain stable and low molecular weight biomimetic molecules which are able to catalyze the dismutation of superoxide anion and therefore provide a suitable alternative to SOD in clinical application. A variety of low molecular weight copper (II) complexes, including those of anti-inflammatory drugs, were prepared and studies as SOD mimics [7–17].

Catechol oxidase is a copper containing enzyme and belongs to polyphenol oxidases. It catalyzes exclusively the two-electron oxidation of catechols in presence of oxygen to the corresponding *o*-quinones [4]. The resulting quinone auto-polymerizes to form brown polyphenolic pigment such as melanins, a process that protects the damaged tissue against pathogens or insects [21]. The catecholase oxidation reaction is of great importance and widely applied in medical diagnosis for the determination of the hormonally active catecholamines, adrenaline, noradrenaline and dopamine [22]. Interest in developing small molecular weight copper (II) complexes as models for copper oxidase enzymes such as catechol oxidase has lead to investigate the catecholase activities of several mononuclear and binuclear copper complexes having different geometries with nitrogen and oxygen donor atoms [1–6,18,19,23,24].

As part of our research on the interaction of copper ion with anti-inflammatory drugs which contain carboxylate group and with biologically active ligands that contain nitrogen atoms, as models for copper proteins, we report here the synthesis,

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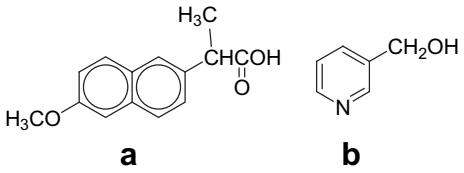

Scheme 1. Molecular structure of naproxen **(a)** and 3-pyridylmethanol **(b)**.

characterization, SOD and catecholase mimetic activities of the binary copper (II) complex of the anti-inflammatory drug naproxen (6-methoxy-α-methylnaphthalene-2-acetic acid) (Scheme 1a), $[Cu_2(Nap)_4]_n$ **(1)**, and its ternary complex with 3-pyridylmethanol (Scheme 1b), $[Cu(Nap)_2(3\text{-pym})_2]_n$ **(2).** The crystal and molecular structure of complex **2** are also reported.

2. Experimental

2.1. Materials

Sodium naproxenate was from Birzeit Pharmaceutical Company (West Bank – Palestine). All other chemicals and solvents used in this study were of high purity grade (Aldrich or Sigma chemicals) and were used without further purification **(1)**.

2.2. Preparation of complexes

2.2.1. Tetrakis(μ-naproxenato) dicopper (II), $[Cu_2(Nap)_4]_n$ (1)

This complex was prepared by adjusting the method described previously and using $CuSO_4.5H_2O$ instead of $CuClO_4.6H_2O$ [25]. A 1.94 g (8.44 mmol) of sodium naproxenate was dissolved in 25 mL of deionized water and the solution was placed in an ice bath. A 0.53 g (2.11 mmol) of $CuSO_4.5H_2O$ dissolved in 8 mL of deionized water was added drop wise to a stirring sodium naproxenate solution over a period of 10–15 min. The mixture was stirred for 2 h. The bluish-green precipitate which formed was suction filtered and washed several times with water and was left on air for dryness. The compound was recrystallized from a mixture of methanol/dichloromethane (1:2).

2.2.2. Bis(naproxenato) bis(3-pyridylmethanol) copper (II),$[Cu(Nap)_2(3\text{-pym})_2]_n$ (2)

A 0.5 g of $[Cu_2(Nap)_4]_n$ was stirred with 3 mL of 3-pyridylmethanol for few minutes, then 7 mL of methanol was added to the mixture which was stirred for 2 h. The blue mixture was filtered and the blue filtrate was left to evaporate under the hood. The pale blue solid which formed was recrystallized from methanol/dichloromethane (1:2) to afford crystals suitable for X-ray determination. *Anal. Calc. for $C_{40}H_{40}N_2O_8Cu$: C, 64.9; H, 5.4; N, 3.8%. Found: C, 64.6; H, 5.5; N, 3.7%.*

2.3. Physical and X-ray measurement

Magnetic susceptibility measurements at 298 K of powdered samples were determined by the Gouy method. Infrared spectra of nujol or hexachlorobutadiene mulls sealed between polyethylene sheets were obtained in the 4000–200 cm^{-1} region with a Perkin–Elmer model 843 infrared spectrophotometer. Electronic spectra of methanol/dichloromethane (1:2) or methanol solutions for **1** and **2,** respectively, were obtained with Hewlett Packard 8433A diode array spectrophotometer. X-band electron spin resonance (ESR) of powdered samples at room temperature were taken with a Varian E-4 spectrometer.

The single crystal X-ray data were collected at 123 K on a Bruker – AXS SMART 2K CCD diffractometer equipped with an Oxford Gryostream crystal cooling system. A full reflection sphere was collected by means of 0.3 ω-scans. The data were collected and reduced using SMART and SAINT Gaussian face-indexing absorption correction [26]. Structure solutions and refinement and graphical illustrations were made with SHELXTL [27]. A summary of crystallographic data is given in Table 1.

2.4. Determination of the superoxide dismutase activity

Superoxide dismutase activity of the complexes **1** and **2** was assayed by using their ability to inhibit the reduction of nitroblue tetrazolium, NBT, at 560 nm by superoxide ions produced by the xanthine – xanthine oxidase system [11,16]. The reaction mixture contained 0.3 mM xanthine, 0.5 mM NBT in 0.05 M phosphate buffer at pH 7.8. The tested complex was dissolved in DMSO and the final concentration of DMSO in the reaction mixture was about 1% in 0.05 M phosphate buffer at pH 7.8. The reaction was started by the addition of xanthine oxidase in the amount needed to yield an absorbance change of 0.03–0.04 unit per minute at 560 nm in the absence of the copper complex. The SOD mimetic activity of the copper complex at 25 °C was evaluated from the absorbance decrease at 560 nm comparing to the blank (the reaction mixture without the copper complex). The concentration of complex required to yield 50% inhibition of NBT reduction (the IC_{50} value) was determined from a plot of percentage inhibition *versus* copper complex concentration.

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

Formula	$C_{40}H_{40}N_2O_8Cu$	*d*calcd, g cm^{-3}	1.402
fw	740.28	*F*(000)	387
Cryst. description	Flat prism	μ, mm^{-1}	0.680
Cryst. color	Pale blue	θ range, deg.	2.39–30.61
Temp., K	123(2)	Reflections collected	16 115
Radiation	Mo Kα, 0.71073 Å	Independent reflections	10 544 [R(int) = 0.0186]
Cryst. size, mm^3	0.375 × 0.15 × 0.05	*hkl* limits	−9,9/−11,11/−24,24
Cryst. system	Triclinic	Completeness to theta = 30.61°	99.2%
Space group	P-1	Absorption correction	Numerical
a, Å	6.4888(5)	Max. and min. transmission	0.8227 and 0.9675
b, Å	7.9795(6)	Refinement method	Full-matrix least-squares on F^2
c, Å	17.3829(13)	Data/restraints/parameters	10 544/5/470
α, deg	99.761(2).	Goodness-of-fit on F^2	1.034
β, deg	94.209(2)	Final R indices [I > 2sigma(I)]	$R1 = 0.0329$, wR2 = 0.0737
γ, deg	96.713(2).	R indices (all data)	$R1 = 0.0398$, wR2 = 0.0759
V, $Å^3$	876.93(11)	Largest diff. peak and hole	0.570 and −0.315 e.$Å^{-3}$
Z	1		

Table 2
Magnetic and Spectral Data for the complexes **1** and **2**.

Complex	Magnetic Moment (BM)	$g_{\perp}$	g_{11}	λ_{max} (nm)	ν_{asy} (CO_2) (cm^{-1})	ν_{sy} (CO_2) (cm^{-1})	$\Delta\nu$ (cm^{-1})
1	1.35	2.07	2.35	690 365(sh)	1585	1400	185
2	1.88	2.06	2.27	698	1600	1380	220

2.5. Determination of the catecholase activity

The catecholase activity of the complexes for the air oxidation of 3,5-di-tert-butylcatechol (DTBC) to the corresponding *o*-quinone (DTBQ) was monitored spectrophotometrically at 25 °C by following the increase of the DTBQ bsorption band at 400 nm. A 0.3 mL of methanol solution (1×10^{-3} M) of the copper (II) complex **2** or methanol/chloroform (1:2) for complex **1** (this complex is insoluble in methanol and reasonably soluble in chloroform), previously saturated with oxygen, was added to 2 mL of a methanol solution of 3,5-di-tert-butylcatechol (0.1 M) in 1 cm quartz cell and the absorbance change at 400 nm *versus* time was recorded immediately for the first 15 min.

3. Results and discussion

3.1. Magnetic and spectroscopic results

The magnetic moments, electronic, IR and ESR spectral data are summarized in Table 2. The room temperature magnetic moment for the binary complex **1** is 1.35BM. This subnormal is due to copper – copper magnetic exchange and is comparable to the magnetic moment values of binuclear copper (II) carboxylates of the type $[Cu_2(RCOO)_4]$ or $[Cu_2(RCOO)_4.L_2]$ [18,23,28,29]. The room temperature magnetic moment for complex **2** is 1.88 BM. This value is consistent with the presence of one unpaired electron in mononuclear copper (II) complexes having d^9 electronic configuration [18,23].

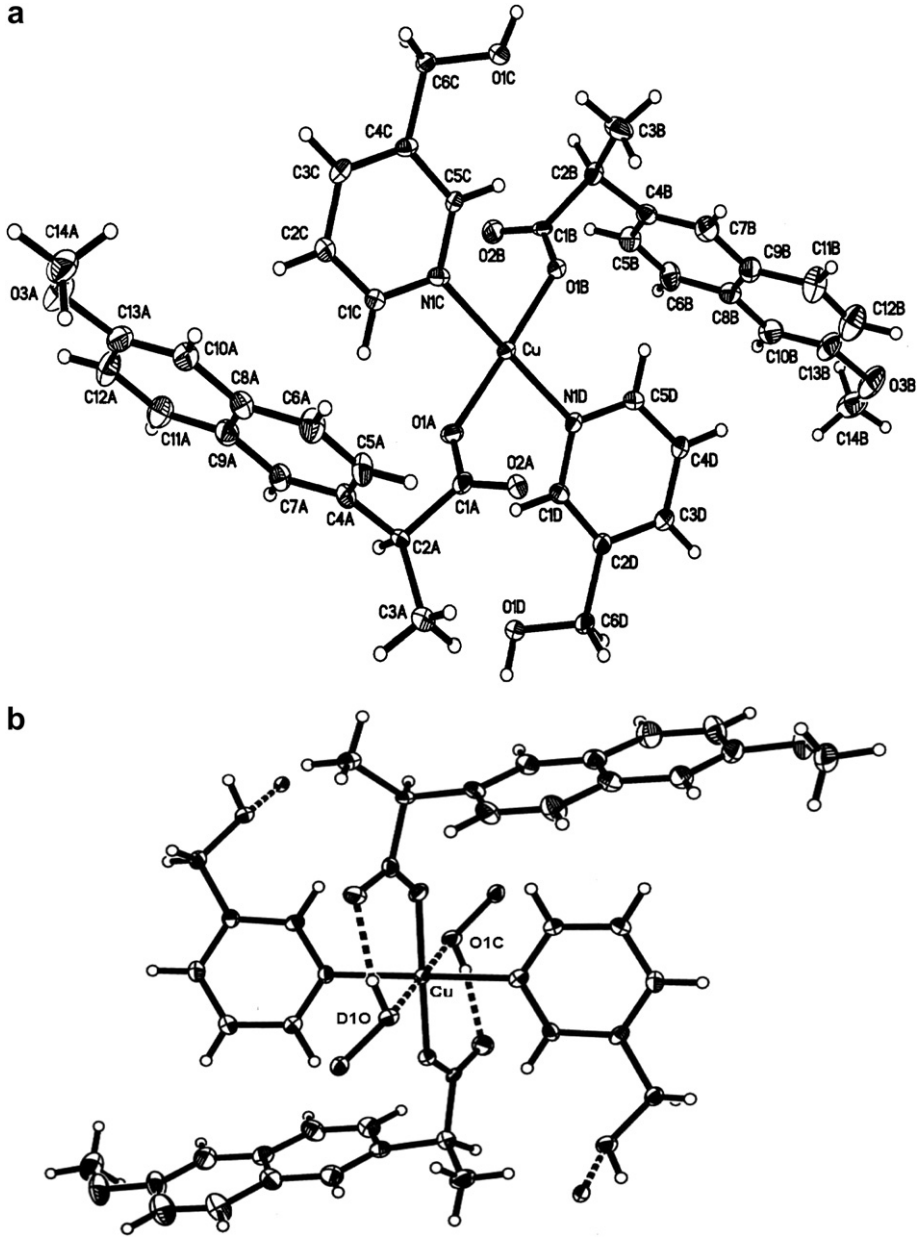

Fig. 1. View of the molecular structure of **2**, showing the atom numbering scheme.

The electronic spectrum of complex **1** in a mixture of methanol/CH_2Cl_2 (1:2) solution exhibited two absorption bands in the 350–850 nm region The band at 690 nm is assigned to copper (II) d–d transitions, where as the shoulder at about 365 nm is referred to the charge transfer band that is considered to be diagnostic of binuclear copper (II) adducts with bridging carboxylates [23,25,28,29]. In the 350–850 nm region the electronic spectrum of complex **2** in methanol solution exhibited one broad absorption band at about 700 nm. This band is assigned to the copper (II) d–d transitions. The position of this band falls within the range expected for mononuclear copper (II) carboxylate complexes that contain a $CuN_2O_2 + O_2$ chromophore in a distorted tetragonal geometry including those of copper (II) carboxylate complexes with 3-pyridylmethanol [18,28,30–32].

The IR spectrum of complex **1** did not show any absorption bands in the 3200–3500 cm^{-1} region characteristic of the coordinated water molecules. The spectrum showed carboxylate stretching frequencies, ν_{asy} (CO_2), at 1585 cm^{-1} and $\nu_{sy}(CO_2)$ at 1400 $cm^{-1}$of the coordinated naproxenate groups, Table 2. The positions of these frequencies and the separation between them $\Delta\nu$ ($\nu_{asy}(CO_2)$–$\nu_{sy}(CO_2)$) of 185 cm^{-1}, when compared to those of sodium naproxenate ($\nu_{asy}(CO_2)$, 1554 cm^{-1}; $\nu_{sy}(CO_2)$, 1395 cm^{-1}; $\Delta\nu$, 159 cm^{-1}) are in the range expected for caboxylate groups that act as bridging bidentate ligands [8,33]. The IR spectrum of complex **2** showed $\nu_{asy}(CO_2)$ and the $\nu_{sy}(CO_2)$ frequencies at 1600 cm^{-1} and 1380 cm^{-1},respectively.The positions of these frequencies and the separation between them, $\Delta\nu$ of 220 cm^{-1}, are in the range expected for monodentate binding mode of the carboxylate groups including those reported for ternary copper carboxylates with 3-pyridylmethanol ligand [30–32]. The strong frequency at 1580 cm^{-1} corresponds to the stretching vibration of C=N of the pyridine ring which is coordinated through the nitrogen atom.

The room temperature ESR spectrum of powdered sample of complex **1** exhibited signals characteristic of triplet state ($S = 1$) of anhydrous copper (II) carboxylates [34]. A broad signal between 3800 and 4600 G, and signals at about 4900 G, at about zero and a very weak signal at about 5600 G. The positions of these signals and the ESR parameters, g_{11} and $g_{\perp}$ (Table 2), are comparable to those reported for anhydrous copper (II) carboxylates having polymeric dinuclear structure [34]. The room temperature ESR sperctrum of the solid state of complex **2** is anistropic and contains g_{11} and $g_{\perp}$ components. The hyperfine coupling constant of Cu(II) ion is partially resolved in the g_{11} region with an A_{11} of about 137 G. The spectral data for this complex are comparable with those reported for tetragonally elongated mononuclear copper(II) complexes having $CuN_2O_2 + O_2$ chromophore, including those reported for copper (II) carboxylate complexes with 3-pyridylmethanol [30–32]. This is supported by the X-ray structural determination of this complex (vide infra).

On the basis of the above spectral and magnetic properties obtained for **1**, a polymeric structure which is built of binuclear units is proposed for the complex. The Cu(II) atoms in each binuclear unit are bridged by four carboxylate groups from naproxenate ions and the units are connected apically through the neighboring carboxylate oxygen atoms. This polymeric structure is generally proposed for anhydrous binuclear copper (II) carboxylates [33–35] including that reported for copper(II) aspirinate whose crystal structure has been reported [35] and confirmed the polymeric dinuclear structure.

3.2. Crystal structure of complex *2*

The X-ray structure of complex **2** is shown in Fig. 1. Crystallographic details are summarized in Table 1, and selected bond lengths and angles are reported in Table 3. The coordination environment of the copper (II) atom is in tetragonal bipyramidal geometry. The Cu atom in the trans-tetragonal plane is coordinated to two oxygen atoms of a pair of unidentate naproxenate anions and two nitrogen atoms from pyridine rings from a pair of neutral 3-pyridylmethanol molecules (Fig. 1a). The axial positions of the tetragonal bipyramid are occupied at longer distances by two methanolic oxygen atoms from the adjacent molecules of the 3-pyridylmethanol ligands (Fig. 1b). The hydrogen atoms of the methanolic hydroxyl groups are forming hydrogen bonds with the non-coordinated oxygen atoms of the naproxenate ions. These hydrogen bonds create a six member metallocyclic rings stabilize the molecular structure (Fig. 1b). The bridging ligand 3-pyridylmethanol between the Cu atoms creates a chain of polymer consists of units of mononuclear Cu (Naproxenate)$_2$(3-pyridylmethanol)$_2$ adduct. The crystal structure of this compound is comparable with those structures reported for other copper (II) carboxylates with 3-pyridylmethanol having $[CuX_2(3\text{-pym})_2]_n$ formula [30–32] [X = carboxylate ligand].

3.3. Superoxide dismutase activity

The superoxide dismutase mimetic activity of the binary complexes **1** and **2**, was measured using the xanthine–xanthine oxidase – nitroblue tetrazolium (NBT) assay system [11,16]. A plot of NBT percent inhibition with an increase in concentration of complex **2** is shown in Fig. 2. The determined IC_{50} values for the complexes under investigation are given in Table 4. In addition, to ascertain the effectiveness of the present complexes as functional

Table 3
Selected bond lengths (Å) and bond angles (°) for complex **2**.

Cu-O1A	1.9434 (18)	O1A–C1A	1.282(3)
Cu-O1B	1.9535 (18)	O1B–C1B	1.275(3)
Cu-N1C	2.066 (2)	N1C–C1C	1.352(2)
Cu-N1D	2.070 (2)	N1D–C1D	1.340(3)
Cu-O1C	2.3969 (19)	C1A–O2A	1.252(3)
Cu-O1D	2.4899 (19)	C1B–O2B	1.237(3)
O1A-Cu-O1B	177.79 (10)	N1C–Cu–O1C	92.66(7)
O1A-Cu-N1C	88.11(8)	N1D–Cu–O1C	90.46(7)
O1B-Cu-N1C	91.66(8)	O1A–Cu–O1D	91.99(7)
O1A-Cu-N1D	91.08(8)	O1B–Cu–O1D	90.19(7)
O1B-Cu-N1D	89.27(8)	N1C–Cu–O1D	87.30(7)
N1C-Cu-N1D	176.73(10)	N1D–Cu–O1D	89.56(7)
O1A-Cu-O1C	87.23(7)	O1C–Cu–O1D	179.21(9)
O1B-Cu-O1C	90.59(7)		

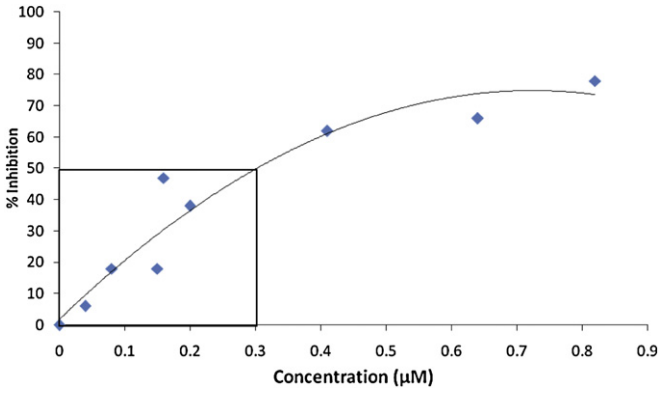

Fig. 2. A plot of percentage of NBT inhibition reduction with an increase in the concentration of complex **1**.

Table 4
Superoxide dismutase activity of copper (II) complexes.

Copper Complex	IC_{50} (μM)	References
$[Cu_2(Nap)_4]_n$, (**1**)	0.30	This work
$[Cu(Nap)_2(3\text{-pym})_2]_n$, (**2**)	0.39	This work
$[Cu_2(Indo)_4(DMF)_2]$[a]	0.23	[15]
	2–25 (solvent dependence)	[13,16]
$[Cu_2(tolf)_4(DMF)_2]$[b]	1.97	[16]
$[Cu(dicl)_2(H_2O)].2H_2O$[c]	2.13	[16]
$[Cu(SalH)_2(H_2O)]0.5H_2O$[d]	1.23	[9]
[Cu(Sal)(phen)][e]	1.01	[9]
$[Cu(SalH)_2(BZDH)_2]$[f]	0.74	[9]
$[Cu(3,5\text{-DTBS})_4(Eth)_4]$[g]	2.69	[17]
Native Cu,Zn − SOD	0.04	[13,15]

Eth = ethanol.
[a] Indo = Indomethacin.
[b] tolf = tolfenamic acid.
[c] dicl = diclofenac acid.
[d] $SalH_2$ = sa1icylic acid.
[e] phen = 1,10-phenanthroline.
[f] BZDH = benzimidazole.
[g] 3,5-DTBS = 3,5-di-tert-butylsalicylate.

SOD mimics, we compared the IC_{50} of several anti-inflammatory drug complexes which were previously determined using the NBT method under the same conditions (Table 4). The IC_{50} values obtained for complexes **1** and **2** indicated that their SOD activities lie in the high activity region of the spectrum exhibited by copper complexes [15]. The IC_{50} value obtained for complex **1** is comparable to that obtained for $[Cu_2(indo)_4(DMF)_2]$ which is considered to be an excellent SOD mimic [15] and is used therapeutically as an oral anti-inflammatory drug in veterinary medicine [13,15]. The mechanism proposed for dismutation of superoxide anions in both the native Cu,Zn–SOD and low molecular weight Cu(II) mimics involved the initial binding of superoxide to Cu(II) ion which allows an electron transfer to occur from $O^{\bullet}_2^-$ to Cu(II) [Eq. (1)] [8]. The Cu (I) complex formed is oxidized back to Cu(II) complex by another molecule of $O^{\bullet}_2^-$ [Eq. (2)]

$$Cu^{2+} + O^{\bullet}_2^- \rightarrow Cu^{+} + O_2, \quad (1)$$

$$Cu^{+} + O^{\bullet}_2^- + 2H^{+} \rightarrow Cu^{2+} + H_2O_2 \quad (2)$$

Some factors were discussed which may involve in the differing dismutation activities shown by different copper complex mimics in vitro [8]. A fast exchange of molecules coordinated axially to copper atom and limited steric hindrance to the approach of the $O^{\bullet}_2^-$ anion are considered essential requirements for the successful binding of the $O^{\bullet}_2^-$The flexibility of copper complex to geometrical arrangement changes, during the redox cycling of Cu(II) ion, which facilitates the interaction of the $O^{\bullet}_2^-$ is also important. The geometry of Cu(II) in SOD enzyme changes from distorted square pyramidal to distorted tetrahedral Cu(I) during dismutation of superoxide anion. In addition, the nature of coordinated ligands to copper is also playing an important role in enhancing the SOD activity of the copper complex mimic. The favorable response of the π-electrons of the coordinated ligands in stabilizing the Cu(II)–$O^{\bullet}_2^-$ interaction and ligands that have groups which are capable to stabilize this interaction through hydrogen bond formation with the coordinated $O^{\bullet}_2^-$ anion, give rise to better SOD mimics [8,36]. The remarkable SOD activity observed for the binary complex **1** is explained in terms of both a fast exchange of axial solvent molecules and a limited steric hindrance to the approach of the superoxide anion in the complex. This polynuclear complex dissociates in solution into its binuclear units, $Cu_2(Nap)_4(Solvent)_2$, since it is weakly connected axially to the neighboring binuclear units. The fast exchange of axial solvent molecules may explain the dependence of the IC_{50} values obtained for binuclear copper indomethacin in different solvents (Table 4) [13,15]. Further, the high superoxide dismutase activities of these binuclear complexes may be also related to possible cooperation of both Cu(II) centers, in close proximity, in free radical binding and electron transfer. One Cu(II) may resemble the role of Zn(II) in the native SOD, through the imidazolate bridge, in controlling the electron density at the redox active Cu(II) [37,38]. The relatively high SOD mimic activity exhibited by complex **2** (Table 4) may be explained in terms of its tetragonally distorted structure having mononuclear units with $CuN_2O_2 + O_2$ chromophore. The relatively weak axial coordination of hydroxyl oxygen of methanolic group of 3-pyridylmethanol are readily dissociated in solution to provide axial sites on Cu(II) for $O^{\bullet}_2^-$ bonding. The dissociation would also facilitate any necessary geometrical changes induced by $O^{\bullet}_2^-$ bonding during catalysis as in the native SOD. The presence of hydroxyl group in the ligand 3-pyridylmethanol, which is hydrogen bonding with the uncoordinated oxygen atom of naproxen carboxylate group in the polymer structure, may assist and stabilizes the axial coordination of the $O^{\bullet}_2^-$ anion to copper atom through hydrogen bond formation, which will result in activity enhancement [36].

3.4. Catecholase activity

Catechol oxidase is a dicopper protein, which catalyzes the two − electron oxidation of *o*-diphenols to the corresponding quinones [4]. The catalytic oxidation of 3,5-di-tert-butylcatechol (DTBC) to the corresponding *o*-quinone (DTBQ) by complex **1** or **2** as biomimetic catalyst has been employed in this study. The *o*-quinone produced (DTBQ) can be followed spectrophotometrically since it is highly stable and shows maximum absorption at about 400 nm in methanol and other solvents (ε = 1900 M^{-1} cm^{-1}). DTBQ is produced in the presence of complex **1** or **2**, but the activity obtained for complex **1,** as mircomoles of substrate produced per mg catalyst per min, is higher than that obtained for complex **2** (1.3 for **1** and 0.35 for **2**). This is consistent with previous studies which showed that binuclear copper complexes, generally, have higher reactivity for the oxidation of catechols to corresponding quinones than mononuclear complexes [4,18,19,23]. The reaction mechanism for the oxidation of catechols by the enzyme catechol oxidase or by synthetic binuclear copper (II) models has been proposed by several studies and involved the binding of substrate to copper (II) atoms. A monodentate asymmetric coordination of catechol to the dicopper (II) core or a simultaneous coordination of the substrate to both copper centers in the bidentate bridging fashion was proposed [4]. The coordinated catechol is oxidized to quinone along with the two-electron reduction of the dicopper centers. Subsequently, the reduced dicopper(I) species reacts with one molecule of oxygen and another molecule of catechol to produce binuclear copper (II) complex and quinone as described below:

$$Cu_2(II)\ \text{dimer} + \text{DTBC} \rightarrow \left[Cu_2(\text{DTBC})^{2-}\right] \quad (3)$$

$$\left[Cu_2(\text{DTBC})^{2-}\right] \xrightarrow{\text{electron transfer}} Cu_2(I) + \text{DTBQ} + 2H^{+} \quad (4)$$

$$Cu_2(I) + O_2 \rightarrow Cu(II)(O_2)^{2-}Cu(II) \quad (5)$$

$$Cu(II)(O_2)^{2-}Cu(II) + \text{DTBC} + 2H^{+} \rightarrow Cu_2(II)\text{dimer} + \text{DTBQ} + 2H_2O \quad (6)$$

In complex **1**, the polynuclear structure is expected to dissociate

in solution into binuclear units and provide axial sites for catechol coordination, and/or the bridging carboxylate groups of naproxen ligands may be used to dehydrogenate the hydroxyl groups of catechol and dicopper (II) catecholate complex is formed (Eq. (3)) which initiates the oxidation process to produce quinone (Eqs. (4,5,6)).

The catalytic mechanism for catechol oxidation by mononuclear Cu(II) complexes has been studied by us and other researchers [4,23,39]. The proposed oxidation reaction proceeds by the following mechanism:

$$\mathrm{Cu(II)complex} + \mathrm{DTBC} \rightarrow \left[\mathrm{Cu(II)(DTBC)^{2-}}\right]\mathrm{complex} \quad (7)$$

$$\left[\mathrm{Cu(II)(DTBC)^{2-}}\right] \overset{\text{electron transfer}}{\leftrightarrow} \left[\mathrm{Cu(I)(DTBSQ)^{-}}\right] \quad (8)$$

$$\left[\mathrm{Cu(I)(DTBSQ)^{-}}\right] \xrightarrow{\text{oxidation}} \mathrm{Cu(II)complex} + \mathrm{DTBQ} \quad (9)$$

For mononuclear copper (II) carboxylate complexes the two carboxylate groups may be used to dehydrogenate the hydroxyl groups of DTBC and Cu(II) – catecholato complex will form (Eq. (7)). An intramolecular electron transfer from the coordinated ligand $DTBC^{2-}$ to copper (II) resulting in the copper (I) semiquinone radical complex, [Cu(I) $(DTBSQ)^{-}$], which is in equilibrium with [Cu (II) $(DTBC)^{2-}$] complex (Eq. (8)). The copper (I) semiquinone radical complex is oxidized by air oxygen to produce Cu (II) complex and DTBQ (Eq. (9)). In complex **2**, the polynuclear structure is expected to dissociate in solution to give the mononuclear units, $Cu(Nap)_2(3\text{-pym})_2$, which interact with DTBC to form the expected Cu (DTBC) $(3\text{-pym})_2$ complex. The two naproxenate groups may dissociate by dehydrogenating the DTBC hydroxyl groups and provide sites on the copper (II) plane for catecholate bonding (Eq. (7)). Optimal intermolecular electron transfer rates would require equatorial coordination to copper (II) in order to maximize overlap between the catecholate donor and the half empty d_{x2-y2} copper(II) orbital, and to form a copper (I) semiquinone species, Cu(I) (DTBSQ) $(3\text{-pym})_2$ (Eq. (8)) which then will be oxidized by an air oxygen to give Cu(II) complex and DTBQ (Eq. (9)).

4. Conclusion

Polynuclear copper (II) complexes of the anti-inflammatory drug naproxen as primary ligand and 3-pyridylmethanol as an auxiliary ligand were synthesized and spectroscopically characterized. The anhydrous binary complex **1** is built of binuclear $[Cu_2(Nap)_4]$ units, linked via Cu – carboxylate oxygen atoms of neighboring units to form polymeric structure. The X-ray crystal structure of the ternary complex **2** consists of mononuclear Cu $(Nap)_2(3\text{-pym})_2$ units in which the copper centers are linked by bridges of 3-pyridylmethanol molecules to form polymeric chain. Both complexes are potent SOD mimic with good potential to be used for therapeutic applications, considering their small molecular weights compared to that of the native enzyme. In addition, these copper complexes are easily formed with bioactive molecules such as naproxen and 3-pyridylmethanol. The complexes showed catecholase mimetic activity for the oxidation of 3,5-di-tert-butylcatechol (DTBC) to the corresponding *o*-quinone (DTBQ). Complex **1** exhibited higher oxidation activity than complex **2** which is consistent with previous studied that showed binuclear copper (II) complexes, generally, have higher reactivity than mononuclear copper (II) complexes for oxidation of catechols to corresponding quinones [4,18,23]. This is may be attributed to different oxidation mechanisms exhibited by the two types of copper (II) complexes and to the fact that the active site of the enzyme catechol oxidase consists of two copper atoms in close proximity with the Cu–Cu distance of 2.9 Å [4].

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Supplementary materials available

Crystallographic data for complex $[Cu(Nap)_2(3\text{-mpy})_2]_n$ (**2**), has been deposited at Cambridge Crystallographic Data Center under the depository number CCDC 761179. Copies of the data can be obtained free of charge upon application to CCDC, 12, Union Road, Cambridge CB2 1EZ, UK. E-mail: deposite@ccdc.cam.ac.uk.

References

[1] A.C. Rosenzweig, M.H. Sazinsky, Curr. Opin. Struct. Biol. 16 (2006) 729–735.
[2] L.M. Mirica, X. Ottenwaelder, T.D.P. Stack, Chem. Rev. 104 (2004) 1013–1046.
[3] S. Itoh, S. Fukuzumi, Acc. Chem. Res. 40 (2007) 592–600.
[4] I.A. Koval, P. Gamez, C. Belle, K. Selmeczi, J. Reedijk, Chem. Soc. Rev. 35 (2006) 814–840 (and references therein).
[5] F.G. Mutti, G. Zoppellaro, M. Gullotti, L. Santagostini, R. Pagliarin, K. K. Anderson, L. Casella, Eur. J. Inorg. Chem. (2009) 554–566.
[6] B. Sreenivasulu, Aust. J. Chem. 62 (2009) 968–979.
[7] Z.A. Siddiqi, M. Shahid, M. Khalid, S. Kumar, Eur. J. Med. Chem. 44 (2009) 2517–2522.
[8] A.L. Abuhijleh, J. Inorg. Biochem. 68 (1997) 167–175.
[9] M. Devereux, D.O. Shea, M.O. Connor, H. Grehan, G. Conner, M. McCann, G. Rosair, F. Lyng, A. Kellett, M. Walsh, D. Egan, B. Thati, Polyhedron 26 (2007) 4073–4084.
[10] V. Balasubramanian, M. Ezhevskaya, H. Moons, M. Neuburger, C. Cristescu, S. V. Doorslaer, C. Palivan, Phys. Chem. Chem. Phys. 11 (2009) 6778–6787.
[11] A.L. Abuhijleh, C. Woods, Inorg. Chem. Commun 5 (2002) 269–273.
[12] J.R.J. Sorenson, Prog. Med. Chem. 26 (1989) 437.
[13] J.E. Weder, C.T. Dillon, T.W. Hambley, B.J. Kennedy, P.A. Lay, J.R. Biffin, H. L. Regtep, N.M. Davies, Coord. Chem. Rev. 232 (2002) 95–126 (and references therein).
[14] T. Fujimori, S. Yamada, H. Yasui, H. Sakurai, Y.I.T. Ishida, J. Biol. Inorg. Chem. 10 (2005) 831–841.
[15] C.T. Dillon, T.W. Hambley, B.J. Kennedy, P.A. Lay, O. Zhou, N.M. Davies, J. R. Biffin, H.L. Regtop, Chem. Res. Toxicol 16 (2003) 28–37 (and references therein).
[16] D.K. Demertzi, A. Galani, M.A. Demertzis, S. Skoulika, C. Kotoglou, J. Inorg, Biochem 98 (2004) 358–364.
[17] G.W. Wangila, K.K. Nagothu, R. Steward III, R. Bhatt, P.A. Iyere, W. M. Willingham, J.R.J. Sorenson, S.V. Shah, D. Portilla, Toxicol.in Vitro 20 (2006) 1300–1312.
[18] A.L. Abuhijleh, J. Inorg. Biochem. 55 (1994) 255–262.
[19] A.L. Abuhijleh, C. Woods, E. Bogas, G. Le Guenniou, Inorg. Chim. Acta 195 (1992) 67–71.
[20] M. Valko, D. Leibfritz, J. Moncol, M.T. Cronin, M. Mazur, J. Teleser, Int. J. Biochem. Cell Biol. 39 (2007) 44–84.
[21] B.J. Dervall, Nature 189 (1961) 311.
[22] D. Meiwes, B. Ross, M. Kiesshauer, K. Cammann, H. Witzel, M. Knoll, M. Borchardt, C. Sandermaier, Lab. Med. 15 (1992) 24.
[23] A.L. Abuhijleh, C. Woods, J. Inorg. Biochem 64 (1996) 55–67.
[24] A.L. Abuhijleh, Polyhedron 15 (1996) 285–293.
[25] A. Trinchero, S. Bonora, A. Tinti, G. Fini, Biopolymers 74 (2004) 120–124.
[26] Bruker. Axs SMART and SAINT. Area Detector Control and Integration Software. Bruker Analytical X – ray Systems Inc., Madison, WI, USA, 1998.
[27] Bruker. AXS SHELXTL Version 6.12. Bruker Analytical X – ray System Inc., Madison, WI, USA, 2001.
[28] C.D. Samara, D.P. Kessissoglou, G.E. Manoussaki, J. Chem. Soc. Dalton Trans. (1990) 959–965.
[29] M. Koman, M. Melnik, J. Moncol, T. Glowiak, Inorg. Chem. Commun 3 (2000) 489–492.
[30] M. Mudra, J. Moncol, J. Svorec, M. Melnik, P. Lonnecke, T. Glowiak, R. Krimse, Inorg. Chem. Commun 6 (2003) 1259–1263.
[31] P. Moncol, D. Segla, M. Miklos, M. Mazur, T. Melnik, M. Glowiak, M. ValkoKoman, Polyhedron 25 (2006) 1561–1566.
[32] J. Kavalirova, Z. Vaskova, J. Maroszova, J. Moncol, M. Koman, T. Lis, M. Mazur, D. Valigura, Z. Anorg, Allg. Chem. 636 (2010) 589–594 (and references therein).
[33] A. Andrade, S.F. Namora, R.G. Woisky, G. Wiezel, R. Najjar, J.A.A. Sertie, D. de O. Silva, J. Inorg. Biochem 81 (2000) 23–27.

[34] B. Kozlevear, I. Leban, M. Petric, S. Petriced, O. Roubeau, J. Reedijk, P. Segedin, Inorg. Chim. Acta 357 (2004) 4220–4230.
[35] F. Garcia, M.A.M. Rojas, E.G. Vergara, S. Bernes, M.A. Quiros, Acta Cryst E59 (2003) m1171–m1173.
[36] R. Martin, A. Fragoso, R. Cao, Supramol. Chem. 15 (2003) 171–175 (and references therein).
[37] P. Starha, Z. Travnicek, R. Herchel, I. Popa, P. Such, J. Vanco, J. Inorg. Biochem 103 (2009) 432–440.
[38] R.C. Marin, G. Alzuet, S. Ferrer, J. Borras, A. Castineiras, E. Monzani, L. Casella, Inorg. Chem. 43 (2004) 6805–6814.
[39] Z.F. Chen, Z.R. Liao, D.F. Li, W.K. Li, X.G. Meng, J. Inorg. Biochem 98 (2004) 1315–1318.