SUPEROXIDE DISMUTASE AND POLYPHENOL OXIDASE BIOMIMETIC ACTIVITIES OF COPPER (II) 3,5-DIISOPROPYLSALICYLATE COMPLEXES WITH HISTAMINE AND CIMETIDINE

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Presented in XXIII.International Conference on Coordination and Bioinorganic Chemistry, June 5-10, 2011, Smolenice-Slovakia, P.21

Abstract - Copper(II) with 3,5-diisopropylsalicylate, $Cu_2(3,5\text{-}DIPS)_4$, is a binuclear tetracarboxylate–bridged complex. This tan solid has been found to have several pharmacological effects which include radioprotectant, radiorecovery, anti-inflammatory, anti-convulsant, anti-diabetic, anti-ulcer, anti-cancer and analgesic activities. Some of these pharmacological activities are enhanced in the presence of ancillary nitrogen donor ligands such as imidazoles, diimines and amines. These pharmacological effects of Cu(II) salicylate type complex may be attributed to the anti-oxidative activities against the reactive oxygen species, especially superoxide radical anion ($O_2^{\cdot-}$), which contribute to the cause of these diseases.

Histamine (Hst) and cimetidine (Cmt) are biologically active ligands and have imidazole and amine nitrogen type ligands and can chelate with metal ions. Cimetidine is a drug that has been used as a powerful histamine H_2-receptor antagonist in the treatment of peptic ulcer. Special interest has been devoted to the interaction of these ligands with

copper(II) ion, since some clinical results support that these interactions play important role in vivo.

We will present our results on the synthesis and spectral characterization of mononuclear ternary complexes of the binary copper(II) 3,5-diisopropylsalicylate, $Cu_2(3,5\text{-}DIPS)_4$, complex with histamine and cimetidine and our results on their activities as superoxide dismutase (SOD) mimics. The polyphenol catalytic activities of these binary and ternary complexes for catecholase oxidation of 3,5-di-*tert*-butylcatechol ($DTBCH_2$) to corresponding *o*-benzoquinone(DTBQ) and for oxidative dealkylation of a hindered 2,4,6-tri-*tert*-butylphenol (TTBP) will also be presented.

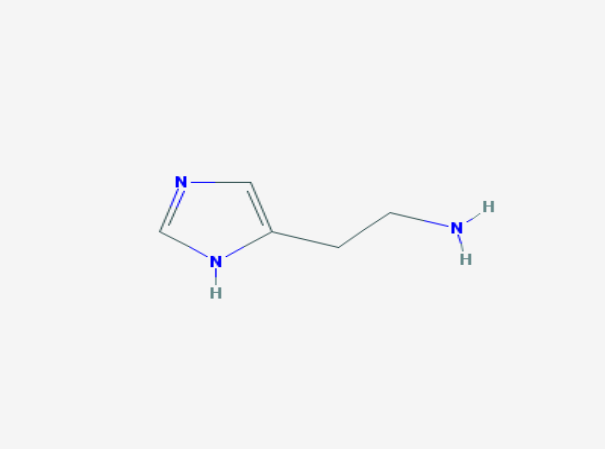
Introduction

Copper(II) with 3,5-diisopropylsalicylate,$Cu_2(3,5\text{-}DIPS)_4$, is a binuclear tetracarboxylate–bridged complex. This tan solid has been found to have several pharmacological effects which include radioprotectant, radiorecovery, anti-inflammatory, anti-convulsant, anti-diabetic, anti-ulcer, anti-cancer and analgesic activities.

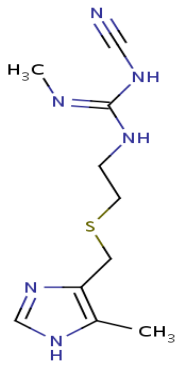
Some of the above mentioned pharmacological activities of $Cu_2(3,5\text{-}DIPS)_4$ are enhanced in the presence of ancillary nitrogen donor ligands such as imidazoles, diimines and amines. These pharmacological effects of Cu(II) salicylate type complex may be attributed to the anti-oxidative activities against the reactive oxygen species, especially superoxide radical anion ($O_2^{\cdot -}$), which contribute to the cause of these diseases.

Histamine (Hst) and cimetidine (Cmt) (structures are shown below) are biologically active ligands and have imidazole and amine nitrogen type ligands and can chelate with metal ions.

Histamine is commonly present in tissues of living organisms and has effects on many physiological and pathological processes. Cimetidine is a drug that has been used as a powerful histamine H_2-receptor antagonist in the treatment of peptic ulcer. Special interest has been devoted to the interaction of these ligands with copper(II) ion, since some clinical results support that these interactions play important role in vivo.



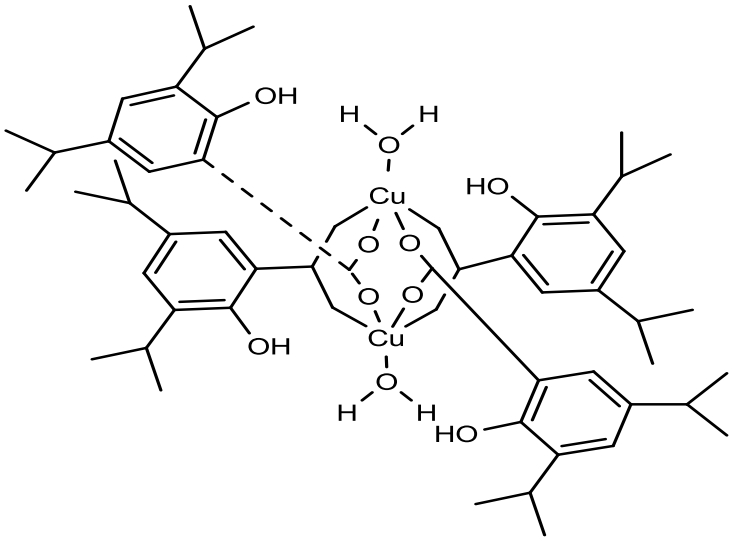
Histamine



Cimetidine

Experimental

Preparation of Cu_2(3,5-diisopropylsalicylate)$_4$,Cu_2(3,5- DIPS)$_4$, complex: Sodium salt of 3,5-diisopropylsalicylate reacts with $CuCl_2.2H_2O$ in water in 2:1 ratio to produce the tan (brown) complex. It has the dimeric paddle wheel structure shown below.



$Cu_2(3,5\text{-}DISP)_4$

Preparation of binary complexes ,Cu(3,5-DIPS)$_2$(histamine)$_2$

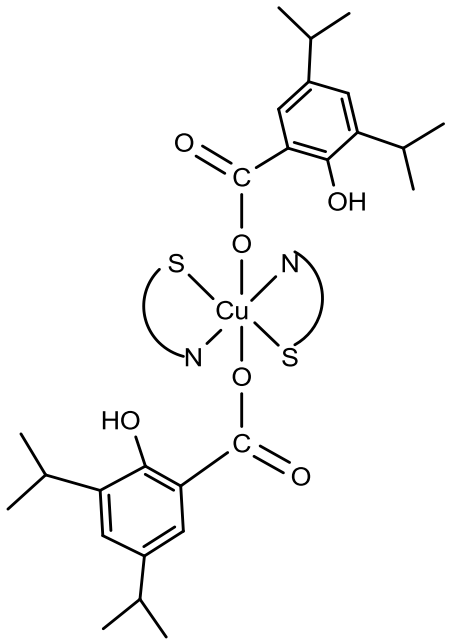
and Cu(3,5- DIPS)$_2$(cimetidine)$_2$: Reaction of binuclear complex Cu_2(3,5- DIPS)$_4$ with histamine or cimetidine (molar ratios are 1:4, respectively) in methanol and re-crystallization of the products from ethanol produced blue crystals of the histamine adduct and dark green crystals of cimetidine adduct .

Spectral and magnetic properties of ternary complexes:

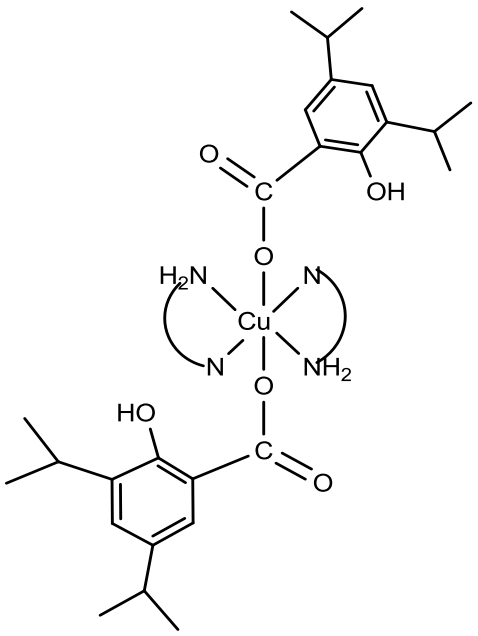
Spectral and magnetic data(magnetic moment, Uv-visible, IR and ESR) data are consistent with the mononuclear structure for the ternary complexes with histamine and cimetidine.

Based on the these initial spectral results copper atom is coordinated by two histamine or cimetidine ligands forming an equatorial plane with two Cu-N of imidazole rings and two Cu-N of NH_2 groups of histamines or two Cu-N of imidazole rings and two Cu-S of cimetidines .Two O atoms from the carboxylate groups of two 3,5-di-isopropylsalicylate anions are coordinated on the elongated axial positions .

The FT-IR spectrum of Cu(3,5- DIPS)$_2$(cimetidine)$_2$ in the 1700- 1000 cm^{-1} region is comparable to that reported for the green complex Cu(**cim**)$_2$(ClO_4)$_2$. In this complex, **cim** acts as a bidendate ligand through N imidazole and S ether atoms.



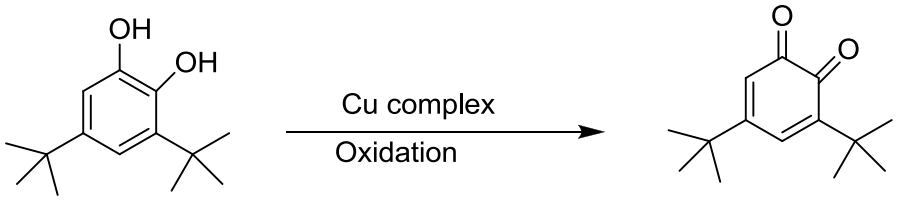
$Cu(3,5\text{-}DISP)_2(Cmt)_2$



$Cu(3,5\text{-}DISP)_2(Hst)_2$

Catecholase mimetic activity.

The catalytic activities of the complexes for the air oxidation of 3,5-di-tert-butylcatechol ($DTBCH_2$) to corresponding o- quinone(DTBQ) were followed spectrophotometrically by monitoring the absorbance increase of DTBQ formation at 400nm (ε=1800 M^{-1} cm^{-1}) as a function of time. Methanol solution of copper complex (0.3 mL of 0.001M) previously saturated with oxygen and 2.0mL of a methanol solution (0.1M) of $DTBCH_2$ were combined in a 1 cm quartz cell at room temperature and the absorbance changes at 400nm were recorded for the first 15 min of the reaction. Complexes **1-4** catalyze the oxidation of $DTBCH_2$ to corresponding *o*-benzoquinone(DTBQ) (Scheme I).



$DTBCH_2$ **DTBQ**

(Scheme 1)

The oxidation activities of the complexes were measured as micromoles of substrate (DBPQ) produced per mg catalyst per min. They are: 0.58 for **1**, 0.57 for **2**, 0.58 for **3.**

It has been accepted now that the catecholase activity of mononuclear copper(II) complexes follows the mononuclear pathway as we and other researchers showed previously. $DTBCH_2$ binds to Cu(II) after its dehydrogenation to form Cu(II) –DTBC complex

followed by an internal electron transfer to form Cu(I)–*o*–semiquinone intermediate species. Oxidation by aerobic oxygen occurs to produce *o*-benzoquinone (DTBQ) and Cu(II) complex as shown in the following equations.

$$\text{Cu(II)complex+DTBC} \longrightarrow [\text{Cu(II)}\ (\text{DTBC})^{2-}] \qquad (1)$$

$$\text{Cu(II)}(\text{DTBC})^{2-}] \quad \text{electron transfer} \quad [\text{Cu(I)}(\text{DTBSQ})^{-}] \qquad (2)$$

$$[\text{Cu(I)}\ (\text{DTBSQ})^{-}] \quad \text{oxidation} \quad \text{Cu(II) complex + DTBQ} \qquad (3)$$

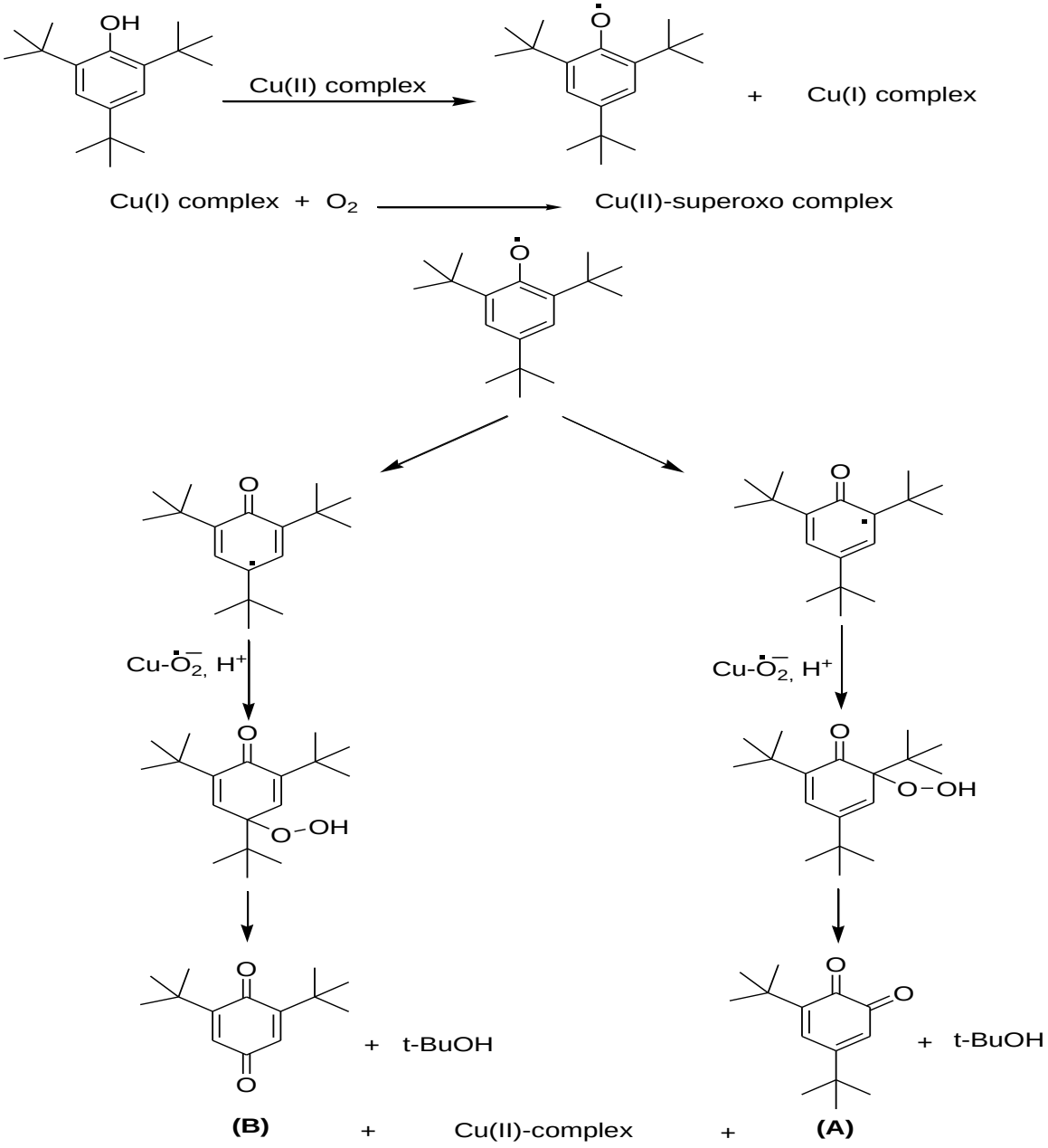
The formation of copper(I)- 3,5-di-t-butyl *o*–semiquinone intermediate during the oxidation process was demonstrated in this study by following the Uv-visible spectral changes of the catalytic reaction mixture. Copper(II) complex (0.02 mmol) and $DTBCH_2$ (0.44 mmol) were mixed in 20 mL degassed methanol under nitrogen and the Uv-visible spectrum of the intense green solution was recorded. Three absorption bands appeared with time: one very broad band with moderate intensity centered at about 770 nm, a second band with less intensity at about 570 nm and a third sharp and very intense band at about 385 nm. The first and third bands at about 770 and 385 nm, respectively, are the ligand $DTBSQ^{-}$ bands (Eq.2) and comparable with those reported previously for Cu(I)-DTBSQ species The second band at about 570 nm is assigned to the catecholato to Copper(II) charge transfer which is in equilibrium with Cu(I)-$DTBSQ^{-}$ (Eq.2) and analogy to assignment made for other Copper(II)-catecholate complexes. When this solution was exposed to aerial oxygen, the bands at 770 nm and 570 nm decayed and the band at 385 nm shifted to ca.400 nm, which is characteristic of DTBQ. Methanol was evaporated from this reaction mixture, the precipitate was extracted with anhydrous diethylether and filterd (copper complexes do not dissolve in ether). Evaporation of the ether filtrate gave

DTBQ, whose IR { ν(CO) =1665 cm^{-1} }, Uv-vis in methanol solution gave band at 400nm, and ^{1}H NMR {$CDCl_3$: δ ppm 1.20(9H), 1.26(9H), 6.20 (1H), 6.98 (1H)} spectra were compared with those of authentic DTBQ.

Oxidative dealkylation of 2, 4, 6-tri-tert-butylphenol (TTBP).

The oxygenation reaction of TTBP with the copper(II)complex as a catalyst and the initial formation of the 2,4,6-tri-tert- butylphenoxyl radical was followed spectrophoto-metrically. An oxygen saturated methanol solution of copper(II) complex (0.3mL of 1×10^{-3}M) was mixed with 2mL (0.05M) of TTBP dissolved in methanol and the formation of phenoxyl radical was followed at 400nm, since TTB phenoxyl radical showed bands in visible region at about 634nm , 400nm and 382nm.

The mechanism for oxygenation dealkylation of TTBP is summarized in Scheme (II) shown below:


OH
Cu(II) complex
O
+ Cu(I) complex
Cu(I) complex + O_2
Cu(II)-superoxo complex
O
Cu-$\dot{O}_2^-$, H^+
Cu-$\dot{O}_2^-$, H^+
O–OH
O–OH
O
O
+ t-BuOH
+ t-BuOH
(B)
+ Cu(II)-complex +
(A)

(Scheme II)

These products [**A** and **B**] are obtained in larger scale, separated and characterized by IR, Uv-visible and NMR spectroscopy. A 0.1mmol of copper complex dissolved in methanol or CH_2Cl_2 and 2mmol (0.525g) of TTBP dissolved in the same solvent, mixed with toluene in 1:1 ratio, was stirred under oxygen for 3 hrs. The reaction mixture was

separated by silica gel column using CH_2Cl_2: n-hexane as eluent solvent (1:20 ratio then increased gradually to 1:2 ratio). In addition to the above mentioned major products (**A**) and (**B**) another 3 products in small quantities were obtained and not identified. The spectral properties of the products (**A**) and (**B**) are summarized below:

IR spectra: products (**A**) and (**B**) exhibited two stretching bands for the carbonyl groups at 1665 and at 1620 cm^{-1} for (**A**) and at 1655 and at 1600 cm^{-1} for (**B**). Uv-visible in methanol solutions showed absorption band at 400nm for (**A**) and at 450nm for (**B**). NMR spectra in $CDCl_3$ for (**A**) exhibited protons chemical shifts (ppm) of tertiary butyl groups at 1.20 (9H) and at 1.26 (9H) and peaks at 6.21 (1H) and 6.96 (1H) for the protons at 3 and 5 positions. NMR spectrum of (**B**) exhibited peaks at 1.27 ppm (18H) for the two tertiary butyl groups and a peak at 6.50ppm (2H) for the two protons at 3 and 5 positions.

Superoxide dismutase activity

The superoxide dismutase mimetic activity of the binary complexes **2** and **3**, was measured using the xanthine-xanthine oxidase – nitroblue tetrazolium (NBT) assay system. A plot of NBT percent inhibition with an increase in concentration of complex **2** is shown in Fig.1. The determined IC_{50} values for the complexes under investigation are given in Table 1. In addition, to ascertain the effectiveness of the present complexes as

functional 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 1). The IC_{50} values obtained for complexes **2** and **3** indicated that their SOD activities lie in the high activity region of the spectrum exhibited by copper complexes. 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 $O_2^{\cdot -}$ to Cu(II) [Eq.1] . The Cu(I) complex formed is oxidized back to Cu(II) complex by another molecule of $O_2^{\cdot -}$ [Eq.2]

$$Cu^{2+} + O_2^{\cdot -} \rightarrow Cu^{+} + O_2, \qquad (1)$$

$$Cu^{+} + O_2^{\cdot -} + 2H^{+} \rightarrow Cu^{2+} + H_2O_2 \qquad (2)$$

Some factors were discussed which may involve in the differing dismutation activities shown by different copper complex mimics in vitro. A fast exchange of molecules coordinated axially to copper atom and limited steric hindrance to the approach of the $O_2^{\cdot -}$ anion are considered essential requirements for the successful binding of the $O_2^{\cdot -}$. The flexibility of copper complex to geometrical arrangement changes, during the redox cycling of Cu(II) ion, which facilitates the interaction of the $O_2^{\cdot -}$ 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) – $O_2^{\cdot -}$ interaction and ligands that have groups which are capable to stabilize this interaction through hydrogen bond formation with the

coordinated $O_2^{\cdot -}$ anion, give rise to better SOD mimics. The SOD activity observed for the binary complex **1** is explained in terms of a fast exchange of axial solvent molecules and 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). The relatively high SOD mimic activity exhibited by complexes **2** and **3** (Table 1) may be explained in terms of its tetragonally distorted structure having mononuclear units with $CuN_2N_2+O_2$ chromophore. The relatively weak axial coordination of carboxylate oxygen atoms of 3,5- di-isopropylsalicylate anions are readily dissociated in solution to provide axial sites on Cu(II) for $O_2^{\cdot -}$ bonding. The dissociation would also facilitate any necessary geometrical changes induced by $O_2^{\cdot -}$ bonding during catalysis as in the native SOD. The higher SOD activity of the histamine adduct may be attributed to the presence of NH_2 group in the histamine adduct (**2**) that is coordinated to copper which can assist and stabilize the axial coordination of the $O_2^{\cdot -}$ anion to copper atom through hydrogen bonding formation, which will result in activity enhancement. The $IC_{50\%}$ values exhibited by complexes **1, 2** and **3** (Table1) fall in the lower end of the range (0.17-29 µM) previously reported for copper(II) complexes with salicylate derivatives, an $IC_{50\%}$ range of which was used therapeutically as anti-inflammatory agents in human and veterinary medicine. In addition, these complexes are potent SOD mimics considering their low molecular weight when compared to that of the native Cu,Zn-SOD

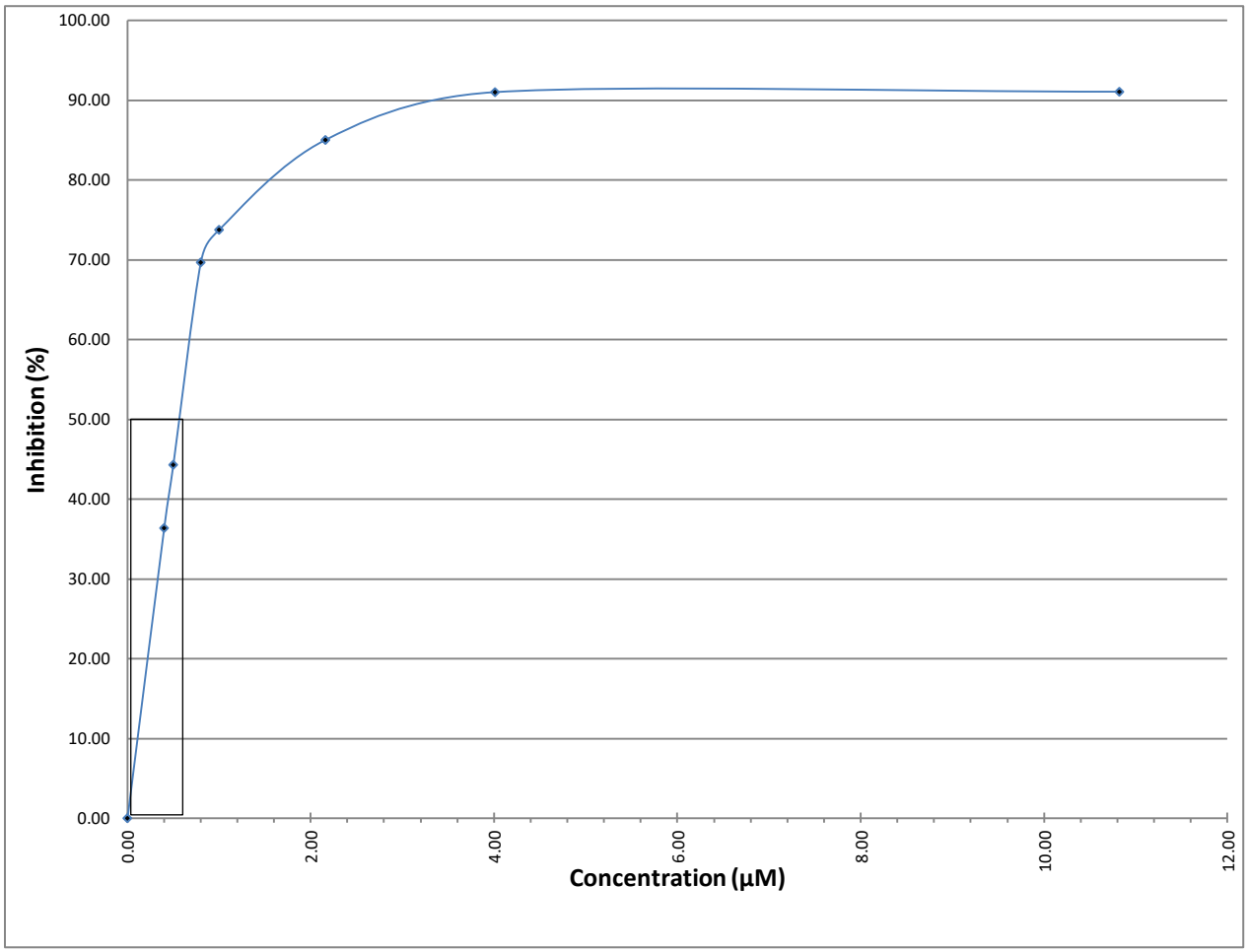


Fig. 1. Percentage of inhibition reduction of NBT against concentration of complex **2**.

Table 1. SOD Mimetic Activity of Cu(II) Complexes

Complex	IC_{50}(μ M)
Cu_2(3,5-DIPS)$_4$	1.0 – 3.0
Cu(3,5-DIPS)$_2$(Hst)$_2$	0.50
Cu(3,5-DIPS)$_2$(Cmt)$_2$	1.70
Cu(Cmt)$_2^{+1/+2}$	0.4-4.0
Cu(Hsal)$_2$(benzimidazole)$_2$	0.74
Cu(sal)(phenanthroline)	1.01
Cu_2 (Salicylate)$_4$	1.30
Cu_2 (aspirinate)$_4$	2.13
Cu_2(Indomethacin)$_4$(DMF)$_2$	0.23
Cu_2(Tolfenamate)$_4$(DMF)$_2$	1.97
Cu,Zn-SOD enzyme	0.04