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Complexes of 2-hydroxyacetophenone semicarbazones: A novel series of superoxide dismutase mimetics

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ABSTRACT

A series of copper(II) and zinc complexes of 2-hydroxyacetophenone semicarbazones have been prepared and evaluated as superoxide dismutase (SOD) mimetics. The SOD-like activity of parent ligands and complexes were determined by the inhibition of nitroblue tetrazolium (NBT) reduction method, using xanthine/xanthine oxidase as the superoxide radical generator. The obtained results indicate that Cu(II) complexes exhibited the most potent SOD-like activities with the IC_{50} values ranging from ca. 0.2 to 4 μM. Among copper complexes, 2-hydroxy-4-methoxyacetophenone semicarbazone analog was the most active compounds (IC_{50} ≈0.2 μM).

Reactive oxygen species (ROS) such as superoxide $O_2^{\cdot -}$ and their products are credited with important roles in the oxidative stress of biological systems.[1] Oxidative stress causes damage to lipid membranes, proteins, lipoproteins, nucleic acids, and many other biomolecules, and leads to the development of a wide range of pathophysiological conditions and serious diseases including cardiac ischemic/reperfusion injury, inflammatory processes, neurodegenerative disorders, atherosclerosis, cancer, or diabetes mellitus.[2–4] Accordingly, the use of superoxide dismutase (SOD) enzymes and modified SODs have been suggested as a useful therapy in combating the action of superoxide radicals in the pathogenesis of inflammatory, degenerative, and metabolic diseases.[5] However, application of natural SODs is limited by their high cost and short plasma half-life, and also some disadvantages in their oral bioavailability, chemical stability, cell permeability and immunogenicity.[6] Therefore, low molecular weight SOD mimics have been potentially studied to overcome these limitations.[7]

Most SOD mimics have been designed with a redox active metal center, similar to the active site metals of the natural SODs, that is, copper(II), which allows the change of metal coordination occurring during the catalytic process. Many low molecular weight complexes of copper and other metals have also been reported to exhibit SOD mimic activities.[8,9] Moreover, copper complexes of amino acid residues, peptides, and salicylate derivatives,[10–13] macrocyclic and tetradentate Schiff-bases[14,15] are known to be useful SOD-like analogs.

Studies of semicarbazones and their metal complexes have been subject of interest because of their chemical and biological properties. In particular, several copper(II) complexes of semicarbazones have been reported to exhibit SOD-like activity. For example, the copper(II) complexes of the Schiff base salicylaldehyde semicarbazone has been reported as a SOD mimic.[16,17] In this Letter, we present preparation and SOD-like activity evaluation of copper(II) and zinc complexes of 2-hydroxyacetophenone semicarbazones.

The copper(II) and zinc complexes of 2-hydroxyacetophenone semicarbazones (**3a–c** and **4a–c**, respectively) were prepared as illustrated in Figure 1. The ligands 2-hydroxyacetophenone semicarbazones **2a–c** were synthesized by reaction of 2-hydroxyacetophenones **1a–c** with semicarbazide hydrochloride.[18] The copper(II) and zinc complexes were prepared by refluxing equimolar amounts of $CuCl_2{\cdot}6H_2O$ or $ZnCl_2$ and the corresponding ligand in EtOH for 2 h. Followed by cooling, a crystalline product was obtained, which were collected, washed with chilled ethanol, and dried under vacuum.[19]

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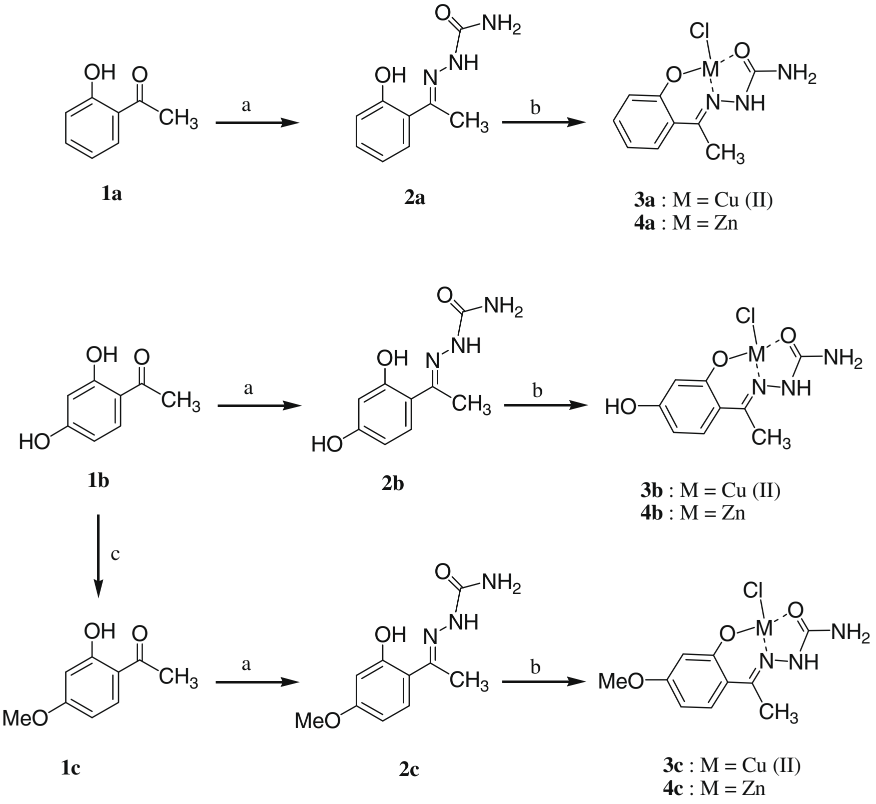


1a **2a** **3a** : M = Cu (II) **4a** : M = Zn

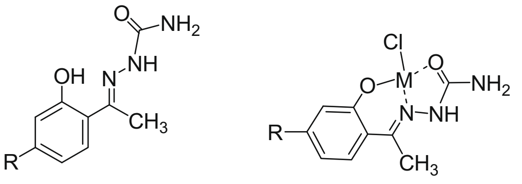
1b **2b** **3b** : M = Cu (II) **4b** : M = Zn

1c **2c** **3c** : M = Cu (II) **4c** : M = Zn

Figure 1. Synthesis of ligands **2a–c**, copper(II) complexes **3a–c**, and zinc complexes **4a–c**. Reagents and conditions: (a) semicarbazide hydrochloride, CH_3COONa, H_2O, rt; (b) $CuCl_2{\cdot}6H_2O$ or $ZnCl_2$, EtOH, reflux, 2 h; (c) MeI, K_2CO_3, acetone, reflux.

Table 1

SOD activity (NBT inhibition) of free ligands **2a–c** and their Cu(II) and Zn complexes (**3a–c** and **4a–c**)



2a-c **3a-c** and **4a-c**

Compound	R	M	IC_{50}[a] (μM)
2a	H		583.09 ± 31.01
2b	OH		>1000
2c	OMe		>1000
3a	H	Cu(II)	4.06 ± 0.35
3b	OH	Cu(II)	0.51 ± 0.24
3c	OMe	Cu(II)	0.2 ± 0.13
4a	H	Zn	151.8 ± 5.05
4b	OH	Zn	90.7 ± 4.3
4c	OMe	Zn	527.01 ± 73.93
$CuCl_2$	–	–	4.77 ± 1.34
SOD[b]	–	–	5.2 units/mL

[a] SOD activity was determined by indirect assay, nitroblue tetrazolium (NBT) method. The IC_{50} value (means ± SD, $n = 3$) is defined as the concentration of test compound that is required to inhibit the reduction rate of NBT by 50%.

[b] Native Cu_2Zn_2-SOD enzyme as positive control.

The SOD activity of parent ligands and complexes were determined by the inhibition of nitroblue tetrazolium (NBT) reduction, using xanthine/xanthine oxidase as the superoxide radical generator.[20] Catalase was also added to remove H_2O_2 from the system. The NBT reduction by $O_2^{\cdot -}$ and formation of blue formazan was spectrophotometrically monitored at 560 nm, estimating its initial rate in the absence, and in the presence of different concentrations of compounds. The IC_{50} values were determined by regression analysis and interpolation of the percent of inhibition versus concentration. The IC_{50} value is defined as the concentration of the compounds that is required to inhibit the reduction rate of NBT by 50%.

The SOD activity of Cu(II) and Zn complexes were compared with those of free ligands, copper(II) chloride and the native Cu_2Zn_2-SOD, as shown by the IC_{50} values in Table 1. The IC_{50} values of the test derivatives indicate that free ligands **2b** and **2c** exhibited no SOD activity (IC_{50} >1000 μM), while remaining ligand **2a** showed some activity. All Cu and Zn complexes were SOD mimics, and showed much greater SOD activity than their corresponding parent ligands. The rage of IC_{50} values for Cu(II) complexes were from ca. 0.2 to 4 μM, while the IC_{50} values of Zn complexes were more than about 90 μM. These results supported the fact that copper is more suitable for the SOD activity. Among copper complexes, methoxy analog of semicarbazone **3c** was the most active compound (IC_{50} value ≈0.2 μM). The introduction of hydroxy or methoxy group at the 4-position of acetophenone system in the copper complexes, increased the SOD activity. Also, the SOD activity of methoxy analogs was higher than that of the hydroxy derivatives in this series. In contrast, substitution of hydroxy or methoxy group in free ligands decreased the SOD activity.

The proposed structure of complexes **3a–c** consists of a neutral, mononuclear four-coordinate copper(II) complex with 2-hydroxyacetophenone semicarbazone ligands providing N,O,O donor atom set while the fourth position is being occupied by the chloride ion. Our knowledge about the redox properties of copper complexes of 2-hydroxyacetophenone semicarbazones as N,O,O donor ligands are limited. Generally, the redox properties of the complexes depend on several factors such as the chelate ring size, axial ligation, degree and distribution of unsaturation and substitution pattern in the chelate ring.[21]

In order to understanding of the redox properties of these copper(II) complexes, cyclic voltammetric measurements of compounds **2a–c** and **3a–c** were carried out.[22] Cyclic voltammograms (CV) of the complexes were recorded and were compared with those obtained with the respective free ligands, in identical experimental conditions. The compounds were scanned at three scan rates. All the cyclic voltammograms are more or less similar with minor variation in the position and appearance of some peaks. The CV obtained for representative ligand **2a**, between −1.2 V and 1.8 V at $v = 100\ \mathrm{mV\ s^{-1}}$, is presented in Figure 2. The complete scan shows only one redox process at $E_{pa} = 0.99$ V that can be related to the oxidation of the phenolic group of the ligand. It is possible to infer that the oxidized species changes through this reaction into a different and electrochemically active compound which is reduced at −0.87 V. Figure 3 shows CV for the copper(II) complex **3a**. In this voltammogram, the redox phenol couple was decreased and a new and high redox peak was appeared at $E_{pa} = 0.49$ and $E_{pc} = -0.41$ for copper center. This shows that the phenol group of ligand is binding with central Cu. The copper center is reduced in one successive two electron transfer reactions (Fig. 3). The first reduction can be attributed to the Cu(II)/Cu(I) process. This couple is found to be quasi-reversible.

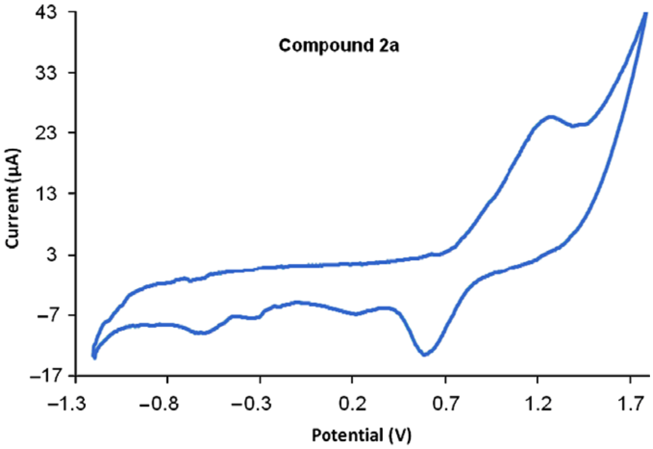


Figure 2. Cyclic voltammogram of free ligand **2a**, 0.01 M at 100 mV s^{-1} scan rate.

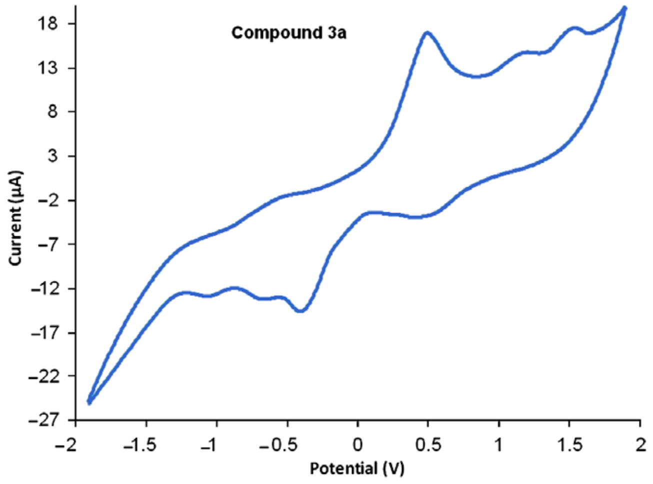


Figure 3. Cyclic voltammogram of copper(II) complex **3a**, 0.01 M at 100 mV s^{-1} scan rate.

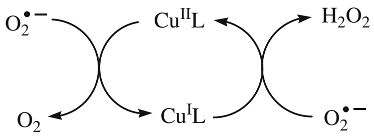


Figure 4. Proposed mechanism for the dismutation of superoxide radicals by copper(II) complexes.

The ratio ($I_p/v^{1/2}$) is practically constant for all the compounds which indicated that is diffusion controlled. For all complexes the couple corresponding to Cu(I/II) redox process can be inferred as quasi-reversible one-electrode processes on the basis of the above observation.

Proposed mechanism for the dismutation of superoxide radicals by copper(II) complexes of 2-hydroxyacetophenone semicarbazones could be explained by the redox cycling of the complexes as shown in Figure 4, in which $Cu^{II}L$ is used as an example for simplicity. The complex $Cu^{II}L$ could be reduced to $Cu^{I}L$ complexes by superoxide radicals. The resulting $Cu^{I}L$ complexes are readily oxidized to $Cu^{II}L$ complexes, while the superoxide radicals are converted to hydrogen peroxide. On the other hand, the coordination geometry is an important factor in characterizing SOD-mimetic complexes. The catalytic cycle of the native Cu_2Zn_2-SOD enzyme, requires the reversible conformational change of the active site from a square planar arrangement of Cu(II) into a distorted tetrahedral form, more adequate to Cu(I).[23] This considerable distortion of the geometry of copper(II) in native Cu_2Zn_2-SOD enzyme is one of the features that enhances the catalytic activity of this enzyme. Therefore, it could be expected that copper(II) complexes exhibiting appreciable square planar distortion should also show high SOD-like activity.[24]

In conclusion, a series of copper(II) and zinc complexes of 2-hydroxyacetophenone semicarbazones have been prepared and evaluated as superoxide dismutase (SOD) mimetics. The catalytic SOD activity of parent ligands and complexes were compared by the NBT reduction bioassay. The obtained results indicate that Cu(II) complexes exhibited the most potent SOD-like activities with the IC_{50} values ranging from ca. 0.2 to 4 µM. Among copper complexes, 2-hydroxy-4-methoxyacetophenone semicarbazone analog was the most active compounds (IC_{50} value ≈0.2 µM). These bioactive compounds have the potential to modulate the biological properties of superoxide anion, thus it is interesting to further investigate their therapeutic efficacy in different biological models of diseases where the overproduction of superoxide radical and/or SOD dysfunction have been implicated.

Acknowledgments

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18. *General procedure for synthesis of compounds* **2a**–**c**: To an aqueous solution of semicarbazide hydrochloride (20.0 mmol) and sodium acetate (25.0 mmol), 2-hydroxyacetophenone derivative (10.0 mmol) was added with stirring resulting in a precipitate. The stirring was continued for 2 h at ambient temperature, after which the precipitate was filtered, washed with distilled water and dried in vacuum to give **2a**–**c**. *Compound* **2a**: yield 68%; mp 218–220 °C; IR (KBr, cm^{-1}): ν_{max} 3344, 3235, 1625, 1597; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.75 (s, 1H, OH), 9.64 (s, 1H, NNH), 7.49 (d, 1H, H-6, J = 6.8 Hz), 7.20 (t, 1H, H-4, J = 8.4 Hz), 6.84 (t, 1H, H-5, J = 6.4 Hz), 6.83 (d, 1H, H-3, J = 8.0 Hz), 6.21 (s, 2H, NH_2), 2.22 (s, 3H, CH_3); MS: m/z (%) 193 (M$^+$, 91), 149 (70), 134 (100), 92 (97). *Compound* **2b**: yield 75%; mp 227–228 °C; (KBr, cm^{-1}): ν_{max} 3483, 3263, 3206, 1680, 1593; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.99 (s, 1H, 2-OH), 9.66 (s, 1H, NNH), 9.47 (s, 1H, 4-OH), 7.30 (d, 1H, H-6, J = 8.4 Hz), 6.27 (dd, 1H, H-5, J = 8.8 Hz and 2.4 Hz), 6.22 (d, 1H, H-3, J = 2.4 Hz), 6.12 (s, 2H, NH_2), 2.17 (s, 3H, CH_3); MS: m/z (%) 209 (M$^+$, 85), 192 (80), 165 (72), 137 (100), 135 (79), 108 (74), 79 (35). *Compound* **2c**: yield 24%; mp 224–225 °C ; (KBr, cm^{-1}): ν_{max} 3503, 3170, 1700, 1593; ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H, OH), 9.55 (s, 1H, NNH), 7.41 (d, 1H, H-6, J = 8.8 Hz), 6.43 (dd, 1H, H-5, J = 8.8 Hz and 2.6 Hz), 6.39 (d, 1H, H-3, J = 2.4 Hz), 6.15 (s, 2H, NH_2), 3.74 (s, 3H, OCH_3), 2.19 (s, 3H, CH_3); MS: m/z (%) 223 (M$^+$, 57), 206 (37), 164 (50), 150 (100), 148 (46), 119 (29), 77 (36).
19. The structures of complexes were identified by their melting points, infrared spectroscopy and elemental analysis. By coordination with copper or zinc, shifts of the carbonyl ν(C=O), azomethyne ν(C=N) and the phenolic ν(C–O) bands, in comparison to the values found in the free 2-hydroxyacetophenone semicarbazone ligands, were observed for the complexes. They were consistent with the tridentate coordination of the semicarbazone derivative ligand through the enolic oxygen atom, the azomethynic nitrogen atom and the phenolic oxygen atom and were in accordance with the published data.[17]
20. *SOD activity*: To a mixture of xanthine oxidase (56 mUnit/mL), catalase (6 units/mL), bovine serum albumin (15% W/V), EDTA (3 mM) and nitroblue tetrazolium (75 mM) in phosphate buffer (10 mM, pH 7), the required concentration of test compound were added. After 30 s, the reaction was started by adding xanthine solution (3 mM) in phosphate buffer. The absorbance of the reaction mixture was monitored spectrophotometrically at 560 nm for 10 min. The SOD activity was calculated base on the rate of absorbance change per minute (ΔA/min). SOD activity was expressed in terms of the IC_{50} of NBT reduction obtained from a linear regression plot of inhibition percent versus concentration.
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