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Research Article

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Manganese(II) complexes of 1,5-diphthalimido-3-azapentane and ethylene diamine as SOD mimetics

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ABSTRACT

The superoxide dismutase (SOD) mimetic were synthesized by complexation of manganese(II) with 1,5-diphthalimido-3-azapentane and ethylene diamine at molar ratios of 1:1:1 (compound 1) and 1:2:2 (compound 2). Their activities were evaluated by measuring inhibition of the photoreduction of nitro blue tetrazolium. Results revealed that both complexes possessed interesting SOD activity (IC_{50}) of 0.16 and 0.09 μM for compounds 1 and 2, respectively. Molecular modeling and quantum chemical calculation of the phthalidimide-based complex was also performed to elucidate the correlation of electrostatic charge of transition metal ions with the SOD activity.

Keywords: SOD, SOD mimetic, phthalidimide, antioxidant, manganese

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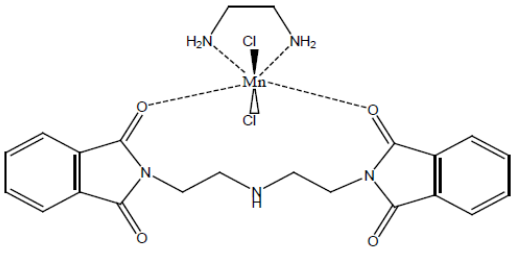
INTRODUCTION

Organometallic synthesis and catalysis has attracted much attention in recent years due to the special chemical reactivity of the transition metals, which can be exploited for a variety of catalytic processes [1]. A number of transition metals are essential trace elements for living organisms, thus it has been utilized in medical applications for treatment of deficiency diseases [2] and used as anticancer [3], antimicrobials [4], and antioxidant [5-7]. Protective and therapeutic roles of SOD have been clinically demonstrated in combating a broad range of diseases [8-10]. However, the applications of SOD as therapeutic agents have been halted by immunological complications and target site accession. Therefore, researchers have shifted their attention to study synthetic SOD mimics that may potentially be developed into pharmaceutical candidates following the notion that removal of superoxide anion can modulate the course of inflammations. Consequently, potential synthetic metal complexes have been shown to possess favorable SOD activity and promising clinical effects [11, 12].

Herein, simple fragments of inorganic molecular scaffolds have been synthesized to obtain a metal-phthalidimide complex. Phthalidimide-based compounds have been reported to exert many biological activities [13, 14]. Moreover, the phthalidimide-based polymers have also been found to be efficient carriers of 5-fluorouracil (5-FU), which possesses anti-tumor activity and reduction of drug toxicity [15]. Thalidomide is a lipophilic drug bearing phthalidimide pharmacophore. Although pharmacological applications of thalidomide have been widely investigated, the molecular mechanism remains dubious. It exhibits pleiotropic effects as a multi-target drug. Thalidomide and derivatives have potential therapeutic value against malaria, tuberculosis, acquired immunodeficiency syndrome (AIDS), breast cancer, colon cancer, prostate tumor, diabetes, rheumatoid arthritis, etc [16-18]. Due to the broad therapeutic value and ease of membrane permeability, phthalidimide-based ligands are promising drug candidates. In the present communication, we report a new potential ligand 1,5-diphthalimido-3-azapentane [19] and its complexation with manganese(II) to obtain compounds **1** and **2** as shown in Figure 1.



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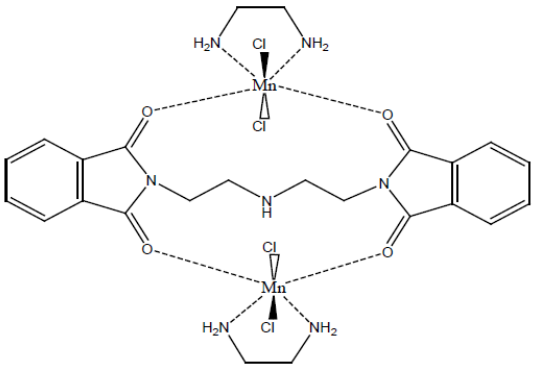


2

Figure 1. Compounds 1 and 2. (1) 1,5-diphthalimido-3-azapentane- $MnCl_2$-ethylene diamine complex (1:1:1 eq). (2) 1,5-diphthalimido-3-azapentane-($MnCl_2$)2-(ethylene-diamine) 2 complex (1:2:2 eq)

EXPERIMENTAL SECTION

Reagents

Diethylenetriamine, phthalic anhydride, ethylene diamine, manganese chloride, Superoxide dismutase, nitro blue tetrazolium (NBT), L-methionine, Triton X 100, riboflavin were purchased from Sigma-Aldrich. All solvents were of analytical or HPLC grade.

Synthesis of the metal complexes

Ligand synthesis: The ligand was synthesized by adding dropwise of diethylenetriamine (27 mL, 0.25 mol) to 74 g (0.5 mol) of phthalic anhydride with vigorous stirring at 180˚C. Steam which developed from the reaction was removed. The temperature was kept at 180 ˚C until solidification of the reaction mixture. Crystallization from xylene afforded 1,5-diphthalimido-3-azapentane: Characterization for Ligand (L) 'H NMR ($CDC1_3$,) δ 2.25 (br s, 1H), 2.95 (t, 4H), 3.75 (t, 4H), 7.65 (s, 8H). IR(KBr) ν 3459.9 (br, NH), 2941.9(CH), 1771.8(w, CO), 1714.6(s, CO), 1465.6, 1436.2, 1396.7, 1047.1(C-N), 720.0 cm^{-1}.

Synthesis of **1** and **2**: The 1,5-diphthalimido-3-azapentane ethylene diamine-manganese complex was synthesized by dissolving 1,5-diphthalimido-3-azapentane (0.364 g, 1 mmol) in methanol (14 mL) followed by heating to homogenize the solution at 60 ˚C. Manganese chloride (0.125 g, 1 mmol) in methanol (1 mL) was added to the solution and stirred for 30 min. Ethylene diamine (0.067 mL, 1 mmol) was added to the prepared solution mixture and stirred for 1 h at 60˚C. After incubation, solvent was removed under vacuum to give dark-brown products.

IR spectra 1: IR(Neat) ν 3382.1(br, NH), 3080(CH), 1771.6(w, CO), 1711.1(s, CO), 1633.7, 1556.1, 1398.3, 1017.7(C-N), 720.6 cm^{-1}

IR spectra 2: IR(KBr) ν 3420.5(br, NH), 3080(CH), 1772.8(w, CO), 1708(s, CO), 1635.9, 1438.2, 1399.7, 1034(C-N), 722.5 cm^{-1}.

Catalysis of superoxide dismutation

The 1 and 2 complexes were tested for SOD activity using previously described method [20-22]. The SOD activity of manganese complex was assayed by measuring the inhibition of the photoreduction of nitro blue tetrazolium (NBT). A stock assay solution was prepared by mixing 27 mL of HEPES buffer (50 mM, pH 7.8), 1.5 mL of L-methionine (30 mg mL^{-1}), 1 mL of NBT (1.41 mg mL^{-1}) and 750 µL of Triton X-100 (1 wt%). To 1 mL of the stock solution in a microcentrifuge tube was added 5 mg of varying amount of 1 and 2 and 10 µL riboflavin (44 µg mL^{-1}). After vortexing, the mixture was illuminated for 7 min under a Classic Tone lamp (60 W) in a light box. Visible absorbance at 550 nm was measured using a standard UV-VIS spectrophotometer. The extent of inhibition for NBT photoreduction was calculated as: $(Abs_{cont} - Abs)/Abs_{cont} \times Abs_{cont}$ represents the absorbance value at 550 nm obtained from the metal-free ligand (L). In this regard, the SOD activity was inversely related to the amount of formazan formed. The IC_{50} values were obtained from the plot of % reduction inhibition versus manganese complex concentration. In a control experiment, metal-free ligand was used to give a background visible absorbance value.

Computational analysis

The calculations were performed using the quantum chemical software Spartan'04 (Spartan'04, Wavefunction, Inc., Irvine, CA.). Molecular mechanics and semi-empirical PM3 were used to obtain the proposed structure of metal complexes. The geometry of the metal complexes was pre-optimized using Merck Molecular Force Field (MMFF). The structure was then subjected to symmetry optimization and full geometry optimization using the semi-empirical PM3 method. The energies of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) were obtained for correlating the electron density distribution to the activity of the metal center and ligand moieties. In addition, electrostatic and Mulliken charges of Mn and dipole moment (µ) of the complexes were calculated.

RESULTS AND DISCUSSION

The manganese complexes were synthesized by treatment of manganese chloride with phthalidimide and ethylene diamine ligands to obtain complexes **1** and **2**. The SOD activities of **1** and **2** were evaluated using previously described method [20-22]. Results revealed SOD activity (IC_{50}) of 120 µg mL-1 (0.16 µM) and 71 µg mL-1 (0.09 µM) for complexes **1** and **2**, respectively (Fig. 2).

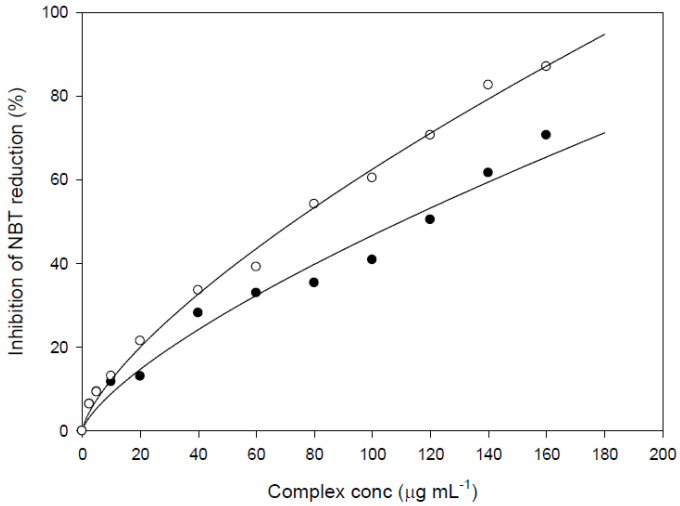
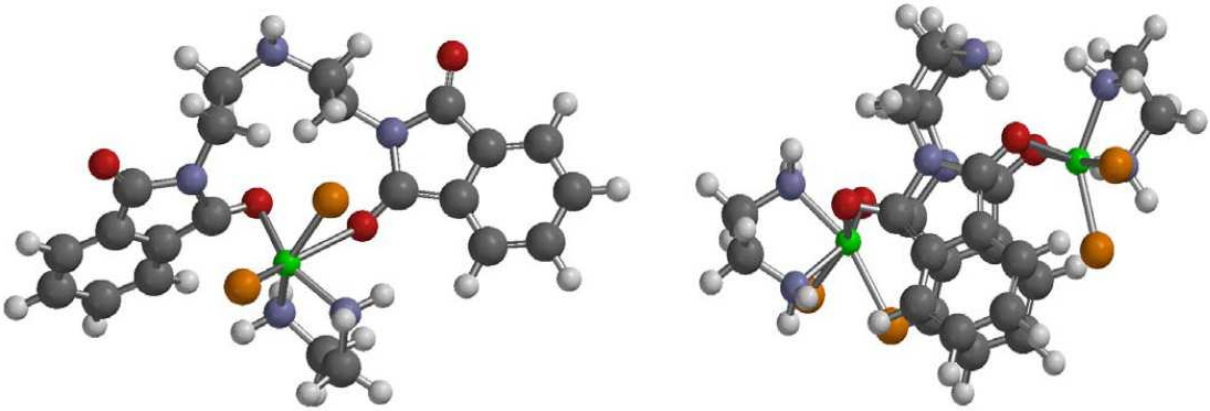


Figure 2. Inhibition of photoreduction of NBT by increasing the amount of 1 (●) or 2 (○)

Interestingly, complex **2** was found to provide SOD activity of nearly two-fold greater than complex **1**. This can be attributed to the fact that complex **2** has two manganese atoms in its structure, therefore, confer electron donating ability to scavenge superoxide anions (Fig. 3).



1 **2**

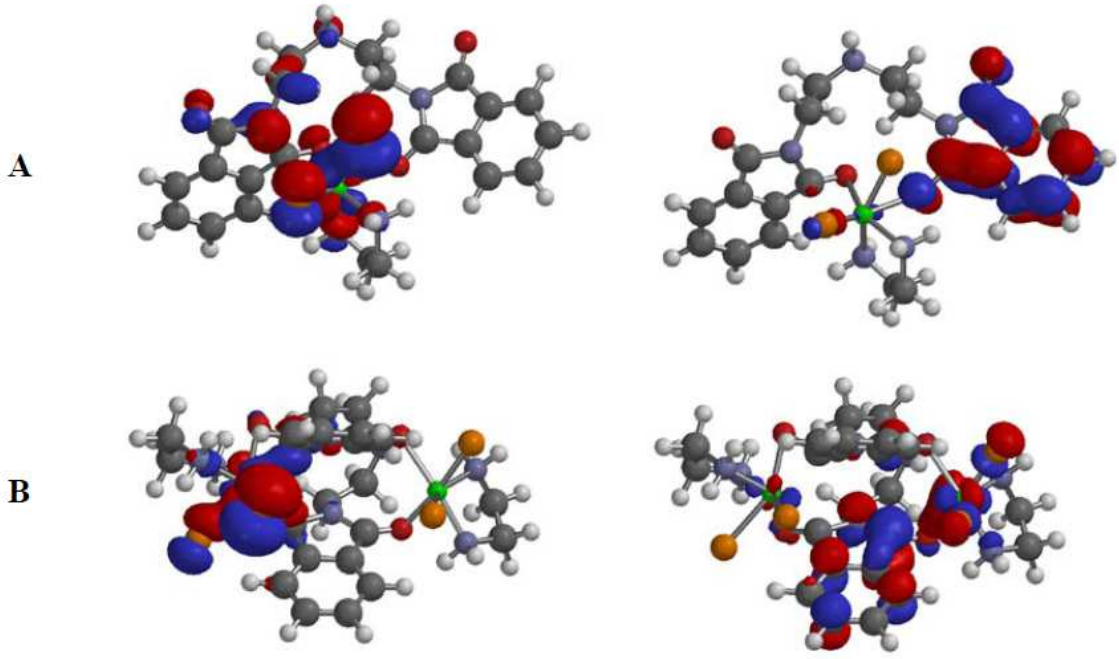
Figure 3. Ball and stick model of 1 and 2, after energy minimization with semi-empirical PM3

To elucidate the structure-activity relationship, we performed molecular modeling and quantum chemical calculations. The molecular properties derived from quantum chemical calculations are shown in Table 1.

Table 1. Molecular properties of complex 1 and 2 derived from quantum chemical calculation

Compounds	Electrostatic charge	Mulliken charge	μ	HOMO	LUMO
Complex 1	0.422[a]	-0.813[a]	14.8	-8.23	-1.25
Complex 1	0.422[b]	-0.813[b]	14.8	-8.23	-1.25
Complex 2 Mn_1[a]	0.714[c]	-0.834[c]	11.21	-7.36	-0.62
Complex 2 Mn_1[b]	0.679[d]	-0.834[d]	11.18	-7.37	-0.62
Complex 2 Mn_2[a]	0.068[e]	-0.740[e]	11.21	-7.36	-0.62
Complex 2 Mn_2[b]	0.115[f]	-0.740[f]	11.18	-7.37	-0.62

[a] *Charge of Manganese ion in complex 1 that was subjected to symmetry optimization.*
[b] *Charge of Manganese ion in complex 1 that was subjected to full geometry optimization.*
[c] *Charge of first Manganese ion in complex 2 that was subjected to symmetry optimization.*
[d] *Charge of first Manganese ion in complex 2 that was subjected to full geometry optimization.*
[e] *Charge of second Manganese ion in complex 2 that was subjected to symmetry optimization.*
[f] *Charge of second Manganese ion in complex 2 that was subjected to full geometry optimization.*



A

B

Figure 4. HOMO/ LUMO for complex 1 (panel A) and complex 2 (panel B)

The molecular properties of the metal complexes displayed non-significant difference when they were subjected to

symmetry or full geometry optimization. Computational investigation on complex **1** shows that the highest occupied molecular orbital (HOMO) is situated in the vicinity of the manganese (Mn) atom, while the lowest unoccupied molecular orbital (LUMO) is situated in the phthalidimide moiety. This character of electrostatic charge is probably attributed to electron delocalization. Superoxide radical ($O_2^{\bullet -}$) is attracted to the Mn center where dismutation takes place, which is followed by electron transfer to the LUMO of phthalidimide moiety. Comparison of the geometry optimization method for complex **2** (Fig. 4) displayed non-significant difference in the values of HOMO, LUMO, and dipole moments. Suggesting that symmetry optimization is suitable for this study as it requires less computational time than full geometry optimization. Computer simulation of complex **2** implies that one of the two Mn atoms possesses higher electrostatic charge than its counterpart. Furthermore, one of the Mn atom in complex **2** exhibited higher electrostatic charge than the Mn atom of complex **1**.

Infrared (IR) spectrum of complexes **1** and **2** showed strong imide carbonyl (CO) absorptions at 1711.1 and 1708.7 cm^{-1}, respectively. As the ligand exhibited the absorption of CO at 1714.6 cm^{-1}. The shift of CO absorptions for **1** and **2** to lower frequency suggests Mn-complex formation. Additionally, the amine (NH_2) absorption of ethylene diamine moiety was not observed. Furthermore, the CH bendings were displayed at 1398.3 (**1**) and 1399.7 (**2**) cm^{-1}, including the out of plane bendings of aromatic moieties showed strong absorptions at 720.6 (**1**) and 722.5 (**2**) cm^{-1}. In our previous study on metallobacitracin [20], it was found that the electrostatic charge of the metal ions was positively correlated with the SOD activity. Likewise, the experimental results in this investigation coincide with the theoretical results in which complex **2** exhibited higher SOD activity than complex 1.

CONCLUSION

In summary we have synthesized a novel SOD mimic based on phthalidimide, a pharmacophore responsible for increasing the lipophilicity. These phthalidomide-based complexes possess interesting SOD activity. Further study is underway to develop novel therapeutic agents for reduction of oxidatively harmful reactive oxygen species.

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