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Synthesis, Structure and Biological Activity of Zn(II) Complex with Tris(benzimidazol-2-yl-methyl)amine Ligand

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A new Zn(II) mononuclear complex with tris(benzimidazol-2-yl-methyl)amine (NTB) was synthesized with stoichiometry of $[Zn(NTB)NO_3]NO_3 \cdot DIPY \cdot DMF$ (DIPY: 4,4'-dipyridyl). The complex was characterized by elemental analysis, UV and IR spectra. The crystal structure was determined by using X-ray diffraction analysis. The crystal structure indicates that four N atoms and one O atom coordinate to zinc ion to construct a distorted trigonal-dipyramid configuration. Three nonprotonated N atoms from imidazole groups are in the equatorial plane, one alkylamino N atom and one O atom from NO_3^- in the axial directions. The biological activity assay shows that this complex presents certain biological activity by means of pyrogallol autoxidation and it can be called a model compound of superoxide dismutase (SOD).

Keywords Zn(II) complex, tris(benzimidazol-2-yl-methyl)amine, superoxide dismutase (SOD), SOD model compound

Introduction

Since the structures of superoxide dismutase (SOD) which appears to play an important role in both the animal and plant kingdoms to eliminate $O_2^{-\cdot}$ and protect living organisms from toxicity of $O_2^{-\cdot}$ [1], exhibit that most of the coordinated atoms around metal ions are from imidazole groups of histidines which are the primary ligands in distorted coordination geometry[2-4] in SOD, an important step should be designed to synthesize the model compounds of SOD in which ligands should contain imidazole groups. We have utilized tris(benzimidazol-2-yl-methyl)amine and bis(benzimidazol-2-yl-methyl)amine as the ligands to synthesize model compounds and analyzed their crystal structures.[5-8]

In order to elucidate the properties of SOD model compounds, we report here the synthesis, structure and the result of activity assay of a new Zn(II) complex with tetradentate ligand tris(benzimidazol-2-yl-methyl)amine. In this paper the relationship between the structure and biological activity is discussed.

Experimental

Preparation of $[Zn(NTB)NO_3]NO_3 \cdot DIPY \cdot DMF$

$Zn(NO_3)_2 \cdot 10H_2O$ (0.01 mol) was dissolved in methanol, 0.01 mol of tris(benzimidazol-2-yl-methyl)-amine (NTB) prepared according to the method reported by Marabella *et al.*[9] was dissolved in methanol and added to the above solution with stirring. Then the mixture was continuously stirred for 8 h at room temperature. After standing, concentrating and filtering, the crude product was recrystallized in methanol to afford light-yellow powder, $[Zn(NTB)NO_3]NO_3 \cdot CH_3OH$ (**A**). Anal. calcd for $C_{25}H_{25}N_9O_7Zn$ (**A**): C 47.76, H 3.98, N 20.02; found C 47.64, H 3.73, N 20.29. UV-vis (EtOH) λ_{max}: 278.90, 272.00, 242.20, 215.10 nm. By means of pyrogallol autoxidation,[5,10] the biological activity of complex **A** was measured, $pI_{50} = 3.70$. 2 mmol of light-yellow powder **A** was dissolved in 10 mL of methanol, 4,4'-dipyridyl (DIPY, 1 mmol) dissolved in 5 mL of methanol was added into the above solution. The mixture was stirred for 8 h at 50 ℃, then distilled and cooled. The precipitate was collected by filtration and washed with methanol, then recrystallized from methanol solution (methanol : H_2O = 2 : 1, *V* : *V*) to afford light-yellow powder, $[Zn(NTB)NO_3]NO_3 \cdot DIPY \cdot CH_3OH$ (**B**). Anal. calcd for $C_{35}H_{33}N_{11}O_7Zn$ (**B**): C 53.56, H 3.89, N 19.64; found C 53.60, H 3.90, N 19.66. Some light-yellow powder of **B** was dissolved in DMF solution, after several days the single crystals of $[Zn(NTB)NO_3]NO_3 \cdot DIPY \cdot DMF$ ($C_{37}H_{36}N_{12}O_7Zn$) were obtained. Anal. calcd for $C_{37}H_{36}N_{12}O_7Zn$: C 53.79, H

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4.39, N 20.35; found C 53.81, H 4.42, N 20.32; UV-vis (EtOH) λ_{max}: 214.3, 245.00, 273.60, 280.50 nm; IR (KBr) ν: 3116 (N—H), 1624 (C=C), 1474, 1454 [C=N (imidazolyl)], 1280 [C—N (imidazolyl)], 1380 [C—N (alkylamino)] cm^{-1}. By means of pyrogallol autoxidation the biological activity of the complex $C_{37}H_{36}N_{12}O_7Zn$ was measured, $pI_{50}=4.21$.

Physical measurement

Elemental analysis, UV-vis and IR (KBr) spectrometry were performed with Perkin-Elmer CHN Elemental Analyzer, TU-1221 Ultraviolet Spectrometer and TJ270-30 Infrared Spectrometer, respectively.

X-ray diffraction experiment

The size of the light yellow crystal of $[Zn(NTB)NO_3]NO_3 \cdot DIPY \cdot DMF$ for diffraction experiment is 0.30 mm×0.20 mm×0.20 mm. A total of 7975 reflections were collected on a Brucker AXS Smart-1000 CCD diffractometer by using graphite-monochromated Mo Kα radiation ($\lambda=0.071073$ nm) with ω and θ scan mode in the range of $1.32° \leqslant \theta \leqslant 25.03°$. All reflections were corrected by Lp factor. 4844 observed reflections with $I \geqslant 2\sigma(I)$ were used in the structure analysis and refinements. The crystallographic parameters are as follows : $C_{37}H_{36}N_{12}O_7Zn$, $M_r=826.16$, triclinic, $a=0.9856(3)$ nm, $b=1.2655(4)$ nm, $c=1.5965(5)$ nm, $\alpha=81.714(5)°$, $\beta=76.601(5)°$, $\gamma=81.504(5)°$, $V=1.9032(10)$ nm^3, $D_c=1.442$ Mg/m^3, $Z=2$, $F(000)=856$, $\mu=0.712$ mm^{-1}, space group $P\bar{1}$.

The structure was solved by direct method and Fourier synthesis method. The positions of hydrogen atoms were determined theoretically. Full matrix least-squares refinements of nonhydrogen atoms with anisotropic thermal parameters and hydrogen atoms with isotropic thermal parameters converged to final $R=0.0424$, $wR=0.0936$, $S=1.013$, $(\Delta/\sigma)_{max}=0.075$, $(\Delta\rho)_{max}=417$ e/nm^3, $(\Delta\rho)_{min}=-381$ e/nm^3. All variables in refinements were 514.

Quantum chemistry calculation

With the Pentium Ⅳ computer, we carried out the quantum chemistry calculation of the complex $C_{37}H_{36}N_{12}O_7$ molecule with B3LYP method at LanL2DZ level using Gaussian 98 program. The atomic coordinates used in the calculation were obtained from the crystal structure data. 93 atoms, 594 basis functions, 1583 primary Gaussian functions, 205 α electrons and 205 β electrons were included in the calculation. In this calculation, the total charge was zero, the multiplicity was one, and after 10 cycles of computation, the energy value reached −72418.147912 eV.

Results and discussion

Discription of the crystal structure

The atomic coordinates and equivalent isotropic temperature factors for non-hydrogen atoms are listed in Table 1, selected bond lengths and bond angles in Table 2. A perspective drawing of the complex $C_{37}H_{36}N_{12}O_7Zn$ is shown in Figure 1 and Figure 2 indicating the packing diagram of the complex $C_{37}H_{36}N_{12}O_7Zn$.

The crystal structure of the complex $C_{37}H_{36}N_{12}O_7$ shows that one stoichiometric molecule is composed of one $[Zn(NTB) \cdot NO_3]^+$, one NO_3^-, one DMF and one DIPY molecule. One alkylamino N atom and three non-protoned N atoms of imidazolyls on NTB and one O atom of NO_3^- are coordinated to zinc ion. N(11), N(13) and N(15) lie in the equatorial plane with Zn ion in the center above the plane by 0.0491 nm toward the O(1) atom. In the axial direction the bond length of Zn(1)—N(17) (0.2441(2) nm) is longer than that of Zn(1)—O(1) (0.2028(2) nm); in the equatorial plane the average bond length of Zn—N is 0.2039 nm. The deviation of the bond angle [O(1)-Zn(1)-N(17), 168.13(8)°] from the normal trigonal dipyramid is 11.87º. Other bond angles around Zn are in the range of 75.51°—120.38°. From above analysis the coordinated center structure is considered as a distorted trigonal dipyramid configuration which is similar to that of other complexes of NTB.[6,7,11,12]

The ligand of NTB molecule presents a rigid tripodal configuration with N(17) on the top and three benzimidazolyl planes as three legs. The dihedral angles between benzimidazolyl plane 1 (leg 1, containing C(1x) atoms) and benzimidazolyl plane 2 (leg 2, containing C(2x) atoms), plane 1 and benzimidazolyl plane 3 (leg 3, containing C(3x) atoms), plane 2 and plane 3 are 120.83°, 127.79° and 120.16° respectively. The coordinated NO_3^- is in the middle of these three planes. In the structure of the complex, zinc ion located in the coordinated place is surrounded by three benzimidazolyl groups and one coordinated NO_3^-. DMF molecule situates at the bottom of leg 2 (plane 2) and the non-coordinated NO_3^- is on the side of the planes 1 and 2. DIPY likely lies above the planes 1 and 3, especially closer to the plane 3. Because the non-coordinated DIPY adjust its conformation easier to suit lower system energy, its dihedral angle (41.68º) of two pyridyl planes is larger than that (32.50º) of the coordinated DIPY.[13] The intermolecular hydrogen bonds among non-coordinated N atoms of NTB, N atoms of DIPY and O atoms of non-coordinated NO_3^- strengthen the stability of the molecule and crystal.

The closest distance between atoms of DIPY and atoms on planes 1 and 3 is 0.3554 nm [C(2)⋯C(37)], so the channels are formed and surrounded by DIPY and two planes (1 and 3) in the crystal. We can suppose that because DIPY exists in the complex $C_{37}H_{36}N_{12}O_7$, there are enough space channels in the structure for $O_2^{-\cdot}$ to move close to the metal ion and further coordinate to it, so the biological activity of the complex $C_{37}H_{36}N_{12}O_7$ is higher than that of complex **A**.

Table 1 Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($nm^2 \times 10^3$) of the complex $C_{37}H_{36}N_{12}O_7Zn$

Atom	*x*	*y*	*z*	U_{eq}[a]	Atom	*x*	*y*	*z*	U_{eq}[a]
Zn(1)	9350(1)	6865(1)	6912(1)	0.35(1)	C(32)	11864(3)	6247(2)	7612(2)	0.38(1)
N(5)	9048(3)	8475(2)	5483(2)	0.51(1)	C(33)	13593(3)	7199(2)	6989(2)	0.42(1)
O(1)	8610(2)	8268(2)	6290(1)	0.57(1)	C(34)	14841(3)	7666(3)	6743(2)	0.57(1)
O(2)	10019(3)	7879(2)	5095(2)	0.70(1)	C(35)	14877(4)	8542(3)	6127(2)	0.59(1)
O(3)	8458(3)	9255(2)	5102(2)	1.04(1)	C(36)	13720(3)	8942(3)	5765(2)	0.50(1)
N(17)	9833(2)	5272(2)	7907(2)	0.37(1)	C(37)	12486(3)	8464(2)	5999(2)	0.42(1)
N(11)	7921(2)	7107(2)	8046(2)	0.39(1)	C(38)	12431(3)	7582(2)	6630(2)	0.36(1)
N(12)	6665(3)	6506(2)	9323(2)	0.47(1)	N(4)	5169(3)	4568(2)	8717(2)	0.54(1)
C(11)	8530(3)	5184(2)	8567(2)	0.47(1)	O(4)	5637(3)	5008(2)	7974(2)	0.71(1)
C(12)	7720(3)	6273(2)	8654(2)	0.39(1)	O(5)	3994(3)	4935(2)	9125(2)	0.65(1)
C(13)	6904(3)	7945(2)	8349(2)	0.41(1)	O(6)	5864(3)	3793(2)	9044(2)	0.83(1)
C(14)	6638(3)	9004(2)	7995(2)	0.48(1)	O(7)	6766(4)	6772(3)	2130(3)	1.33(1)
C(15)	5563(4)	9646(3)	8455(2)	0.62(1)	N(3)	5495(4)	7976(3)	2987(2)	0.68(1)
C(16)	4766(4)	9252(3)	9249(3)	0.72(1)	C(40)	4173(6)	8356(4)	3518(3)	1.14(2)
C(17)	5011(4)	8215(3)	9615(2)	0.64(1)	C(41)	6695(6)	8497(4)	2987(4)	1.36(2)
C(18)	6099(3)	7564(3)	9153(2)	0.45(1)	C(42)	5647(6)	7157(4)	2559(3)	0.87(1)
N(13)	8926(2)	5633(2)	6359(2)	0.38(1)	N(1)	9094(3)	11742(2)	7319(2)	0.58(1)
N(14)	8910(3)	3888(2)	6305(2)	0.45(1)	N(2)	10394(4)	7050(2)	10195(2)	0.73(1)
C(21)	10175(3)	4398(2)	7357(2)	0.43(1)	C(1)	9904(4)	10860(3)	7057(2)	0.64(1)
C(22)	9325(3)	4637(2)	6681(2)	0.39(1)	C(2)	10206(4)	9955(3)	7602(2)	0.60(1)
C(23)	8180(3)	4421(2)	5689(2)	0.40(1)	C(3)	9645(3)	9919(2)	8483(2)	0.42(1)
C(24)	7521(3)	4050(3)	5128(2)	0.52(1)	C(4)	8785(4)	10824(2)	8759(2)	0.51(1)
C(25)	6850(4)	4816(3)	4606(2)	0.56(1)	C(5)	8555(4)	11698(3)	8166(2)	0.56(1)
C(26)	6843(3)	5912(3)	4649(2)	0.55(1)	C(6)	9916(4)	8938(2)	9081(2)	0.44(1)
C(27)	7507(3)	6280(3)	5196(2)	0.48(1)	C(7)	8848(4)	8558(3)	9740(2)	0.55(1)
C(28)	8196(3)	5514(2)	5727(2)	0.38(1)	C(8)	9130(5)	7627(3)	10267(2)	0.70(1)
N(15)	11337(2)	6953(2)	7030(1)	0.34(1)	C(9)	11420(5)	7426(3)	9574(3)	0.66(1)
N(16)	13193(3)	6349(2)	7602(2)	0.47(1)	C(10)	11229(4)	8342(3)	9011(2)	0.54(1)
C(31)	11020(3)	5467(2)	8247(2)	0.45(1)					

[a] U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

Table 2 Selected bond lengths (nm) and angles (°) of the complex $C_{37}H_{36}N_{12}O_7Zn$

Zn(1)—O(1)	0.2028(2)	Zn(1)—N(15)	0.2032(2)
Zn(1)—N(11)	0.2050(2)	Zn(1)—N(17)	0.2441(2)
Zn(1)—N(13)	0.2036(2)		
O(1)-Zn(1)-N(11)	92.67(9)	N(11)-Zn(1)-N(15)	111.41(10)
O(1)-Zn(1)-N(13)	108.15(10)	N(11)-Zn(1)-N(17)	75.51(9)
O(1)-Zn(1)-N(15)	109.36(10)	N(13)-Zn(1)-N(15)	120.38(9)
O(1)-Zn(1)-N(17)	168.13(8)	N(13)-Zn(1)-N(17)	75.93(9)
N(11)-Zn(1)-N(13)	111.26(10)	N(15)-Zn(1)-N(17)	76.65(8)

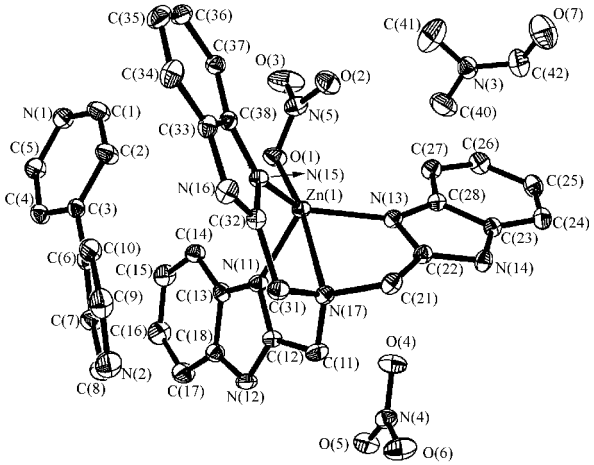

Figure 1 Perspective drawing of the complex $C_{37}H_{36}N_{12}O_7Zn$.

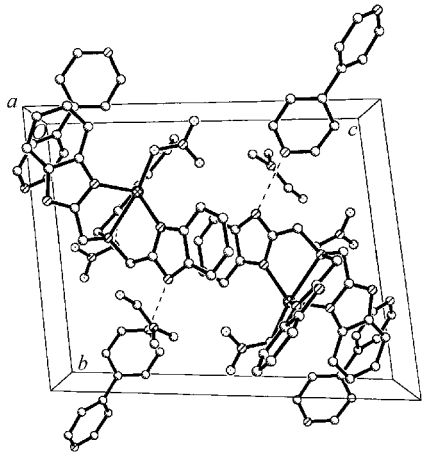

Figure 2 The packing diagram of the complex $C_{37}H_{36}N_{12}O_7Zn$.

Brief analysis for quantum chemistry calculation

According to molecular orbital theory, the frontier orbital and nearby molecular orbitals are the most important factors to the bioactivity.[14,15] The highest occupied molecular orbital (HOMO) and nearby occupied molecular orbitals are prior to donating electrons, but the lowest unoccupied molecular orbital (LUMO) and nearby unoccupied molecular orbitals are prior to accepting electrons. The components and high proportion (%) of frontier molecular orbitals are listed in Table 3.

The components of most atoms on plane 1 are larger in the HOMO, while the components of all the atoms on plane 3 and DIPY are larger in the LUMO. This tells us that the atoms on the plane 1 are prior to donating electrons, which means that they could repel $O_2^{-\cdot}$; while the atoms on the plane 3 and DIPY could draw electrons of $O_2^{-\cdot}$ effectively. From those mentioned above we can say that there is an active area composed of the atoms on the planes 1, 3 and DIPY. This conclusion is in accordance with the result of crystal structure in which there are channels composed of the same atoms. Meanwhile in the LUMO the component of Zn(II) ion is higher than that in HOMO and the net charge of Zn(II) is 1.512378 a.u. (a.u.=|e|). We can conclude that Zn(II) ions are susceptible to drawing $O_2^{-\cdot}$ radicals through the active channels and making them coordinate to Zn(II) ions, so the complex $C_{37}H_{36}N_{12}O_7$ which we called the model compound of SOD presents higher activity than the complex **A**.

Table 3 The frontier molecular orbital components and proportion (%) of the complex $C_{37}H_{36}N_{12}O_7Zn$

Atom	HOMO/%	Atom	LUMO/%	Atom	LUMO/%
N(11)	7.36	N(15)	3.69	N(1)	3.68
N(12)	0.01	N(16)	3.38	N(2)	6.46
C(11)	0.49	C(31)	2.11	C(1)	1.02
C(12)	11.99	C(32)	12.38	C(2)	2.46
C(13)	23.82	C(33)	8.37	C(3)	3.01
C(14)	4.55	C(34)	1.93	C(4)	0.46
C(15)	5.63	C(35)	3.50	C(5)	4.89
C(16)	22.53	C(36)	7.69	C(6)	9.62
C(17)	3.91	C(37)	0.41	C(7)	4.23
C(18)	14.12	C(38)	1.09	C(8)	1.36
Zn(1)	0.11	Zn(1)	0.67	C(9)	2.07
				C(10)	2.21

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