Synthesis, Characterization, Crystal Structures, and Urease Inhibition of Copper(II) and Zinc(II) Complexes Derived from Benzohydrazones

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Abstract

A new copper(II) complex [Cu(L1)(NCS)(CH3OH)] (1) and a new zinc(II) complex [ZnCl2(HL2)] · CH3OH (2), derived from 4-bromo-N'-(pyridin-2-ylmethylene)benzohydrazide (HL1) and 4-methoxy-N'-(pyridin-2-ylmethylene)benzohydrazide (HL2), were prepared and characterized by elemental analysis, IR and UV-Vis spectroscopy and single crystal X-ray diffraction. The hydrazone HL1 coordinates to the Cu atom in enolate form, while the hydrazone HL2 coordinates to the Zn atom in carbonyl form. Single crystal structural analyses indicate that the hydrazones coordinate to the metal atoms through the pyridine N, imino N, and enolate/carbonyl O atoms. The Cu atom in complex 1 is in square pyramidal coordination, and the Zn atom in complex 2 is in trigonal-bipyramidal coordination. The inhibitory effects of the complexes on Jack bean urease were studied, which show that the copper complex has strong activity on urease.

Keywords: Hydrazone; copper complex; zinc complex; crystal structure; urease inhibition

1. Introduction

Urease (EC 3.5.1.5; urea amidohydrolase) is a biunimolecular nickel-dependent hydrolase enzyme, which can be synthesized by numerous organisms, including plants, bacteria, algae, fungi, and invertebrates, and occurs widely in animal and soil.1 Urease enzyme catalyzes the decomposition of urea into ammonia and carbon dioxide in high efficiency,2 with the rate of catalyzed reaction 10^{14} times higher than the non-catalyzed reaction.3 The enzyme possesses harmful effects on both human health and fertile soil.4 In recent years, Schiff base complexes are reported to have interesting urease inhibitory activities,5 especially the copper complexes with Schiff bases or hydrazones are promising types of lead structures as urease inhibitors.6 As a continuation of the work on the exploration of new urease inhibitors, a new copper(II) complex [Cu(L1)(NCS)(CH3OH)] (1) and a new zinc(II) complex [ZnCl2(HL2)] · CH3OH (2), derived from 4-bromo-N'-(pyridin-2-ylmethylene)benzohydrazide (HL1) and 4-methoxy-N'-(pyridin-2-ylmethylene)benzohydrazide (HL2; Scheme 1), were prepared and studied on their urease inhibition activity.

2. Experimental

2.1. Materials and Measurements

All reagents and solvents were of commercially available reagent grade quality and were used without further purification. HL1 was synthesized according to the literature method.7 Jack bean urease was purchased from Sigma.

Scheme 1. HL1 and HL2
ma-Aldrich. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer. IR spectra were recorded on a Jasco FT/IR-4000 spectrometer as KBr pellets in the 4000–400 cm$^{-1}$ region. UV-Vis spectra were recorded on a Perkin-Elmer Lambda 900 spectrometer. $^1$H NMR and $^{13}$C NMR spectra were recorded on a 500 MHz Bruker Advance instrument. The urease inhibitory activity was measured on a Bio-Tek Synergy HT microplate reader. Single crystal structures were determined by Bruker Smart 1000 CCD area diffraction.

2. 2. Synthesis of HL$^2$

2-Pyridinecarboxaldehyde (0.01 mol, 1.07 g) and 4-methoxybenzohydrazide (0.01 mol, 1.66 g) were dissolved in methanol (100 mL). The mixture was stirred at reflux for 1 h and the solvent removed by distillation at reduced pressure. The solid product was re-crystallized from methanol to give colorless crystals. Yield: 1.72 g (68%). Characteristic IR data (KBr, cm$^{-1}$): 3227 (NH), 1645 (C=O), 1607 (C=N). UV–Vis data (methanol, $\lambda$/nm): 305, 370. Anal. Calcd for C$_{14}$H$_{13}$N$_3$O$_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.71; H, 5.25; N, 16.32%. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ (ppm): 11.96 (s, 1H, NH), 8.62 (d, 1H, PyH), 8.47 (s, 1H, C=NH), 7.99-7.89 (m, 4H, PyH+ArH), 7.42 (q, 1H, PyH), 7.10 (d, 2H, ArH), 3.84 (s, 3H, CH$_3$). $^{13}$C NMR (DMSO-$d_6$, 126 MHz) $\delta$ (ppm): 162.70, 162.11, 153.37, 149.43, 147.36, 136.76, 129.62, 125.16, 124.19, 119.74, 113.71, 55.40.

2. 3. Synthesis of the Complex 1

HL$^1$ (1.0 mmol, 0.30 g) was dissolved in methanol (20 mL), to which Cu(ClO$_4$)$_2$ · 6H$_2$O (1.0 mmol, 0.37 g) and ammonium thiocyanate (1.0 mmol, 0.076 g) dissolved in methanol (20 mL) were added dropwise. The mixture was stirred for 10 min at room temperature and filtered. The filtrate was kept in air for a few days, to form crystals suitable for single crystal X-ray diffraction. The crystals were isolated by filtration. Yield: 0.18 g (39%). Characteristic IR data (KBr, cm$^{-1}$): 3450 (OH), 2043 (NCS), 1602 (C=NH), 1447, 1372, 1161, 1070, 952, 860, 535. UV–Vis data (methanol, $\lambda$/nm): 270, 370. Anal. Calcd for C$_{15}$H$_{13}$BrCuN$_4$O$_2$S: C, 39.44; H, 2.87; N, 12.26. Found: C, 39.27; H, 2.98; N, 12.41%.

2. 4. Synthesis of the Complex 2

HL$^2$ (1.0 mmol, 0.26 g) was dissolved in methanol (20 mL), to which ZnCl$_2$ (1.0 mmol, 0.14 g) dissolved in methanol (20 mL) was added dropwise. The mixture was stirred for 10 min at room temperature and filtered. The filtrate was kept in air for a few days, to form crystals suitable for single crystal X-ray diffraction. The crystals were isolated by filtration. Yield: 0.26 g (61%). Characteristic IR data (KBr, cm$^{-1}$): 3463 (OH), 1672 (C=O), 1604 (C=NH), 1455, 1369, 1276, 1158, 1078, 947, 860, 544, 520. UV–Vis data (methanol, $\lambda$/nm): 305, 380. Anal. Calcd for C$_{15}$H$_{17}$Cl$_2$N$_3$O$_3$Zn: C, 42.53; H, 4.05; N, 9.92. Found: C, 42.71; H, 4.13; N, 9.80%.

| Compound | HL$^2$ | 1 | 2 |
|----------|-------|---|---|
| Formula  | C$_{14}$H$_{15}$N$_3$O$_3$ | C$_{15}$H$_{13}$BrCuN$_4$O$_2$S | C$_{15}$H$_{17}$Cl$_2$N$_3$O$_3$Zn |
| $M_1$    | 273.3 | 456.8 | 423.6 |
| Crystal system | Triclinic | Monoclinic | Triclinic |
| Space group | P$\bar{1}$ | P$2_1$/n | P$\bar{1}$ |
| $a$ (Å)  | 6.6036(12) | 7.3009(5) | 7.8943(4) |
| $b$ (Å)  | 14.6112(11) | 17.3938(13) | 8.3532(5) |
| $c$ (Å)  | 15.2091(13) | 13.7578(10) | 14.7609(9) |
| $\alpha$ (°) | 71.232(2) | 90 | 104.246(1) |
| $\beta$ (°) | 84.436(2) | 96.629(1) | 100.125(1) |
| $\gamma$ (°) | 84.520(2) | 90 | 99.016(1) |
| $V$ (Å$^3$) | 1379.7(3) | 1735.4(2) | 906.16(9) |
| $Z$      | 4 | 4 | 2 |
| $D_1$ (g cm$^{-3}$) | 1.316 | 1.748 | 1.552 |
| $\mu$ (Mo-Ka) (mm$^{-1}$) | 0.095 | 3.696 | 1.668 |
| $F(000)$ | 576 | 908 | 432 |
| Reflections collected | 8135 | 8964 | 4869 |
| Unique reflections | 5104 | 3218 | 3345 |
| Observed reflections ($I \geq 2\sigma(I)$) | 3655 | 2514 | 2995 |
| Parameters | 381 | 221 | 231 |
| Restraints | 6 | 1 | 2 |
| Goodness-of-fit on $R^2$ | 1.041 | 1.039 | 1.060 |
| $R_1$, wK$_2$ ($I \geq 2\sigma(I)$) | 0.0535, 0.1425 | 0.0381, 0.0891 | 0.0256, 0.0658 |
| $R_1$, wK$_2$ (all data) | 0.0775, 0.1609 | 0.0545, 0.0959 | 0.0301, 0.0681 |
| Large diff. peak and hole (eÅ$^{-3}$) | 0.223, –0.249 | 0.977, –0.471 | 0.274, –0.234 |

Table 1. Crystallographic and experimental data for the compounds
2. 5. X-Ray Crystallography

Diffraction intensities for HL\textsuperscript{2} and the complexes were collected at 298(2) K using a Bruker Smart 1000 CCD area diffractometer with MoK\textsubscript{α} radiation (\(\lambda = 0.71073\) Å). The collected data were reduced with SAINT\textsuperscript{8} and multi-scan absorption correction was performed using SADABS\textsuperscript{9}. Structures of HL\textsuperscript{2} and the complexes were solved by direct methods and refined against \(F^2\) by full-matrix least-squares method using SHELXTL\textsuperscript{10}. All of the non-hydrogen atoms were refined anisotropically. The amino and methanol H atoms in the compounds were located from difference Fourier maps and refined isotropically, with N–H and O–H distances restrained to 0.90(1) and 0.85(1) Å, respectively. The remaining hydrogen atoms were placed in calculated positions and constrained to ride on their parent atoms. Crystallographic data for HL\textsuperscript{2} and the complexes are summarized in Table 1. Selected bond lengths and angles are given in Table 2.

2. 6. Urease Inhibitory Activity Assay

The measurement of urease inhibitory activity was carried out according to the literature method\textsuperscript{11}. The assay mixture containing 75 \(\mu\)L of jack bean urease and 75 \(\mu\)L of tested compounds with various concentrations (dissolved in DMSO) was pre-incubated for 15 min on a 96-well assay plate. Acetohydroxamic acid was used as a reference. Then 75 \(\mu\)L of phosphate buffer at pH 6.8 containing phenol red (0.18 mmol L\textsuperscript{-1}) and urea (400 mmol L\textsuperscript{-1}) were added and incubated at room temperature. The reaction time required

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**Table 2.** Selected bond lengths/Å and angles/° for the compounds

|          | HL\textsuperscript{2} | N2–N3 | N2–N3 | N2–N3 |
|----------|------------------------|-------|-------|-------|
| C6–N2    | 1.272(3)               | 1.375(2) |       |       |
| N3–C7    | 1.356(3)               | 1.229(3) |       |       |
| C20–N5   | 1.273(3)               | 1.369(2) |       |       |
| N6–C21   | 1.360(3)               | 1.229(2) |       |       |
| C6–N2–N3 | 116.4(2)               | 118.6(2) |       |       |
| C20–N5–N6| 116.9(2)               | 118.1(2) |       |       |

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**Scheme 2.** The synthetic procedure of the hydrazones and the complexes. X = Br for HL\textsuperscript{1}, OMe for HL\textsuperscript{2}.

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for enough ammonium carbonate to form to raise the pH phosphate buffer from 6.8 to 7.7 was measured by micro-plate reader (560 nm) with end-point being determined by the color change of phenol-red indicator.

3. Results and Discussion

3.1. Chemistry

The synthetic procedure of the hydrazones and the complexes is shown in Scheme 2. The hydrazone HL\textsubscript{2} was prepared by the condensation reaction of equimolar quantities of 2-pyridinecarboxaldehyde and 4-methoxybenzohydrazide. The copper complex was prepared by reaction of equimolar quantities of HL\textsubscript{1}, copper perchlorate, and ammonium thiocyanate in methanol. The zinc complex was prepared by reaction of equimolar quantities of HL\textsubscript{2} and zinc chloride in methanol. Single crystals of HL\textsubscript{2} and the complexes were obtained by slow evaporation of the methanolic solution of the compounds.

3.2. Structure Description of HL\textsubscript{2}

The molecular structure of HL\textsubscript{2} is shown in Figure 1. The compound contains two hydrazone molecules and two water molecules of crystallization. The hydrazone molecules adopt E configuration with respect to the methylenedie unit. The distances of the methylenedie bonds confirm them as typical double bonds. The shorter distances of the C–N bonds and the longer distances of the C=O bonds for the –C(O)–NH– units than usual, suggests the presence of conjugation effects in the hydrazone molecules. The remaining bond lengths in the compounds are within normal values. The dihedral angles between the pyridine and benzene rings are 61.0(3)° for one molecule and 18.4(3)° for the other one. The hydrazone molecules are linked by water molecules through hydrogen bonds of O–H...O and O–H...O (Table 3, and Figure 2).

3.3. Structure Description of the Copper Complex

Molecular structure of the copper complex is shown in Figure 3. The Cu atom is in square pyramidal geometry, with the pyridine N, imino N, and enolate O atoms of the hydrazone ligand, and the thiocyanato N atom located at the basal plane, and with the methanol O atom located at the apical position. The Cu atom deviates from the least-squares plane defined by the four basal donor atoms by 0.242(1) Å. The coordinate bond lengths in the complex

| D–H...A          | d(D–H) | d(H...A) | d(D...A) | Angle (D–H...A) |
|------------------|--------|---------|----------|----------------|
| HL\textsubscript{2} |        |         |          |                |
| O5–H5B–N1       | 0.85(1)| 2.18(2) | 2.982(3) | 157(2)         |
| O5–H5A–O3       | 0.85(1)| 1.99(1) | 2.808(2) | 162(3)         |
| O6–H6B–O1\textsuperscript{i} | 0.85(1)| 1.99(1) | 2.830(3) | 165(3)         |
| O6–H6A–N4\textsuperscript{i} | 0.85(1)| 2.19(2) | 2.981(3) | 155(3)         |
| N3–H3–O6        | 0.86   | 2.04    | 2.863(3) | 161(3)         |
| N6–H6C–O5\textsuperscript{ii} | 0.86   | 2.07    | 2.898(2) | 162(3)         |

Symmetry codes: (i) –1 + x, y, z; (ii) 1 + x, y, z; (iii) –x, –y + 2, –z + 2.

Table 3. Hydrogen bond distances (Å) and bond angles (°) for the compounds

Figure 1. Molecular structure of HL\textsubscript{2}, showing the atom-numbering scheme. Displacement ellipsoids for non-hydrogen atoms are drawn at 30% probability level.
Figure 2. Molecular packing structure of HL, viewed along the $a$ axis. Hydrogen bonds are shown as dashed lines.

Figure 3. Molecular structure of 1, showing the atom-numbering scheme. Displacement ellipsoids for non-hydrogen atoms are drawn at 30% probability level.

Figure 4. Molecular packing structure of 1, viewed along the $b$ axis. Hydrogen bonds are shown as dashed lines.
are comparable to those observed in copper(II) complexes with hydrazine ligands.6a,13

In the crystal structure of the complex, two complex molecules are linked through intermolecular hydrogen bonds of O–H⋯N (Table 3), to form a dimer (Figure 4).

3. 4. Structure Description of the Zinc Complex

Molecular structure of the zinc complex is shown in Figure 5. The Zn atom is in trigonal bipyramidal geometry, with the imino atom of the hydrazone ligand, and two chloride atoms located at the basal plane, and with the pyridine N and carbonyl O atoms located at the axial positions. The Zn atom deviates from the least-squares plane defined by the three basal donor atoms by 0.129(1) Å. The coordinate bond lengths in the complex are comparable to those observed in zinc(II) complexes with hydrazine ligands.14

In the crystal structure of the complex, the complex molecules are linked through intermolecular hydrogen bonds of O–H⋯O (Table 3), to form chains along the b axis (Figure 6).

3. 5. Biological Study

The percent inhibition of the compounds at concentration of 100 μmol L⁻¹ on jack bean urease is summarized in Table 4. The hydrazones and the zinc complex have weak activity. However, the copper complex showed strong
urease inhibitory activity, with IC$_{50}$ value of 1.4 ± 0.8 μmol L$^{-1}$. As a comparison, acetylamidoxamic acid (AHA) was used as a reference drug with the percent inhibition of 84.3 ± 3.9, and with IC$_{50}$ value of 37.2 ± 4.0 μmol L$^{-1}$. Thus, the present copper complex is a good model for urease inhibition.

4. Conclusion

In summary, a new hydrazone compound 4-methoxy-N$^2$-(pyridin-2-ylmethylene)benzohydrazide was prepared and structurally characterized. With the hydrazones, a new copper(II) complex and a new zinc(II) complex were obtained. The complexes were characterized by physico-chemical method, and their structures were confirmed by single crystal X-ray determination. The copper complex has strong urease inhibitory activity, which deserves further study to explore novel and efficient urease inhibitors.

Supplementary Data

CCDC 1998916 (HL$^2$), 1547384 (1) and 1813293 (2) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk. The IR and UV-Vis spectra are given in the supplementary information.

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Povzetek

Sintetizirali smo nov bakrov(II) kompleks \([\text{Cu}(L^1)(\text{NCS})(\text{CH}_3\text{OH})]\) (1) in nove cinkov(II) kompleks \([\text{ZnCl}_2(\text{HL}_2)]\cdot\text{CH}_3\text{OH}\) (2) z vezavo 4-bromo-\(N'\)-(piridin-2-ilmetilen)benzohidrazida (\(\text{HL}_1\)) in 4-metoksi-\(N'\)-(piridin-2-ilmetilen)benzohidrazida (\(\text{HL}_2\)) ter ju okarakterizirali z elementno analizo, IR in UV-Vis spektroskopijo ter monokristalno rentgensko difrakcijo. Hidrazon \(\text{HL}_1\) se koordinira na Cu atom v enolatni obliki, medtem ko se hidrazon \(\text{HL}_2\) koordinira na Zn atom v karbonilni obliki. Rentgenska monokristalna analiza razkrije, da se hidrazona koordinirata na kvadratno piramidalno, medtem ko se \(\text{Cu}\) in \(\text{Zn}\) atom koordiniran kvadratno piramidalno, medtem ko se \(\text{Zn}\) atom v kompleksu koordinirana trigonalno bipiramidalno. Proučili smo inhibitorni vpliv kompleksov na ureazo stročnice \(\text{Canavalia ensiformis}\), ki kaže, da ima bakrov kompleks večjo aktivnost na ureazo.