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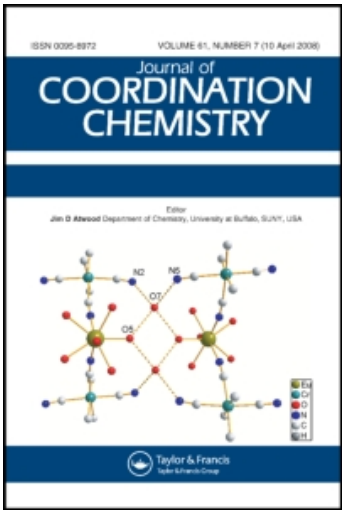

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Synthesis, characterization and antimicrobial activity of a Zn(II) complex with 1-(1H-benzoimidazol-2-yl)-ethanone thiosemicarbazone

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Synthesis, characterization and antimicrobial activity of a Zn(II) complex with 1-(1H-benzoimidazol-2-yl)-ethanone thiosemicarbazone

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A new Zn(II) complex with 1-(1H-benzoimidazol-2-yl)-ethanone thiosemicarbazone $[Zn(NO_3)(H_2O)(C_{10}H_{11}N_5S)]NO_3$ was prepared and characterized by elemental analyses, FT-IR, ^1H NMR spectroscopy, thermogravimetric analysis (TGA), X-ray diffraction (XRD), and single-crystal X-ray diffraction analysis. The coordination geometry of the pentacoordinated zinc is a distorted square pyramid. The antimicrobial activity of the complex was evaluated using a broth micro-dilution method against a panel of human pathogenic Gram positive, Gram negative bacteria and the yeast *Candida albicans*. The best inhibitory effect was observed against *Enterobacter aerogenes* ($MIC = 0.031\,mg\,mL^{-1}$).

Keywords: Zinc complex; Thiosemicarbazone complex; Benzimidazole complex; Crystal structure; Antimicrobial activity

1. Introduction

Thiosemicarbazones have a broad spectrum of biological properties [1] and versatility as ligands from the presence of several potential donors, their flexibility, and their ability to coordinate in either neutral or deprotonated forms [2]. Complexes between metals and thiosemicarbazone ligands exhibit a broad spectrum of biological properties, including antibacterial, antimalarial, antiviral and antineoplastic activities [3]. Benzimidazole and its derivatives have also attracted attention because of their broad spectrum of biological activities [4, 5], including antimicrobial [6, 7], antifungal [8] and as antitumor agents [9–11]. However, there is almost no pharmacological or

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coordinative information about benzimidazole thiosemicarbazone derivatives or complexes.

In this article, we report the synthesis, structural characterization and crystal structure of a new zinc complex of 1-(1H-benzoimidazol-2-yl)-ethanone thiosemicarbazone. In addition, the preliminary *in vitro* antimicrobial activity of the complex is evaluated by a broth microdilution assay. This work was carried out to compare their coordinative behaviors and antimicrobial activities. Zinc was chosen because zinc complexes have antimicrobial effect against bacteria, fungi, and viruses and inhibitory effects against carbonic anhydrases, carboxy peptidases, thermolysin, and alcohol dehydrogenase [12–14].

2. Experimental

2.1. *Materials and instruments*

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All chemicals and reagents were analytical grade, used as received from commercial sources (Sigma-Aldrich and Alfa Aesar). The IR spectra (KBr pellet, 4000–400 cm^{-1}) were recorded on a Perkin Elmer BX-II FT-IR spectrophotometer. Elemental analyses for C, N, S and H were performed on Elementar Vario III EL. ^{1}H NMR spectra were recorded on a Bruker AC 500 MHz instrument in *dmso-d*6. Thermogravimetric analyses were performed on a Linseis Thermowaage L81 Series thermal analysis system by heating (20°C min^{-1}) ca 8 mg of the compound under air in the temperature range 0–800°C. XRD data were collected using a Shimadzu 6000 diffractometer with Cu-Kα radiation at 0.02° s^{-1} step.

2.2. *Synthesis of $[Zn(NO_3)(H_2O)(C_{10}H_{11}N_5S)]NO_3$ complex*

A solution of 0.125 g (0.422 mmol) $Zn(NO_3)_2 \cdot 6H_2O$ in 20 ml of ethanol (95°C) was added dropwise to a solution of 0.098 g (0.422 mmol) of 1-(1H-benzoimidazol-2-yl)-ethanone thiosemicarbazone in 15 ml of ethanol. The resulting solution was stirred under reflux for 2 days. The mixture was cooled and slow evaporation at room temperature gave a pale yellow solid. The product was recrystallized from absolute ethanol (yield 59%, w/w). Anal. Calcd. for $C_{10}H_{12}N_7O_7SZn$: C, 27.27; H, 2.72; N, 22.38; S, 7.29. Found: C, 27.24; H, 2.86; N, 21.98; S, 7.36%. IR (KBr, cm^{-1}): ν(NH2) + ν(NH) + ν(OH) 3305 m, 3246 m, 3145(m); ν(C=N) + δ(NH2) 1615 m, 1547 m; δ(NH) 1522(m); ν(ring) + δ(NCS) 1490(w), 1458(s); ν(N–O) 1319(m); ν(N–O) 1046(w); ν(C=S) + γ(CH) 831(m); ν(N–O) 805(w); ν(N–O) 660(sh); γ(CH) 752(m); δ(ring) 630(w); ^{1}H NMR (dmso-d6, ppm): 12.6 (s, 1H, HN2); 11.80 (s, 1H, HN4); 8.5 (s, 2H, H2N5); 7.70 (d, 1H, HC2); 7.45 (d, 1H, HC5); 7.20 (t, 1H, HC3); 7.12 (t, 1H, HC4); 2.5 (s, 3H, H_3C9); 4.09 (s, broad, 2H, H_2O4).

2.3. *Crystal structure determination*

The crystal and instrumental parameters used in the unit-cell determination and data collection are summarized in table 1. Diffraction measurements were made at room

Table 1. Crystal data and structure refinement details.

Empirical formula	$[Zn(NO_3)(H_2O)(C_{10}H_{11}N_5S)]NO_3$
Formula weight	440.73
Temperature (K)	296(2)
Radiation (λ, Å)	MoKα (0.71073)
Crystal system, space group	Triclinic, $P\bar{1}$
Unit cell dimensions (Å, °)	
a	8.482(2)
b	9.170(2)
c	10.947(2)
α	100.726(12)
β	98.708(12)
γ	95.447(12)
Volume (Å^3)	820.3(3)
Z	2
Calculated density (Mg m^{-3})	1.784
Absorption coefficient (mm^{-1})	1.677
$F(000)$	448
Crystal size (mm^3)	0.240 × 0.140 × 0.100
θ Range for data collection (°)	2.45–30.54
Index ranges	$-12 \le h \le 11$, $-13 \le k \le 13$, $-15 \le l \le 15$
Reflections collected	4973
Independent reflections	3934 $\{R_{int} = 0.0564\}$
Data/parameters	3934/253
Final R indices $[I \ge 2\sigma(I)]$	$R_1 = 0.0437$, $wR_2 = 0.1063$
Goodness of fit on F^2	1.050
Largest difference peak and hole (e Å^{-3})	0.364 and −0.465

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temperature on a CrystalClear X-ray diffractometer using graphite-monochromated Mo-Kα radiation using $\omega/2\theta$ scan mode [15]. Unit-cell dimensions were determined and refined by using the angular settings of 25 automatically centered reflections in the $2.45 \le \theta \le 30.54$ range. The structure was solved by direct methods using SHELXS-97 and refined by full-matrix least-squares techniques on F^2 with SHELXL-97 [16]. Empirical absorption corrections were applied by the multi-scan method via CrystalClear software. All non-hydrogen atoms were refined with anisotropic displacement parameters and hydrogen atoms were included in their idealized positions and refined isotropically except for the water and imidazole nitrogen (N_2) hydrogens were located from difference maps and refined isotropically. An ORTEP drawing [17] of complex with 40% probability displacement thermal ellipsoids and atom-labeling scheme are shown in figure 1.

2.4. *Antimicrobial activity*

The antimicrobial activity of the Zn(II) compound was evaluated by micro-dilution susceptibility [18, 19]. Microbial strains and suspensions were grown as previously described in detail [18]. Initial stock solutions of the test samples (2 mg mL^{-1}) were prepared in 25% (v/v) dimethylsulfoxide (DMSO, Carlo Erba) which was used as starting concentration serially diluted in equal volume of Mueller-Hinton broth (MHB, Merck, Germany) up to 0.008 mg mL^{-1} in a 96-well microtiter plate format. Sufficiently grown microbial suspensions were standardized then to 1×10^8 CFU mL^{-1} using McFarland No: 0.5, also in MHB. 100 µL of each microbial suspension was then added

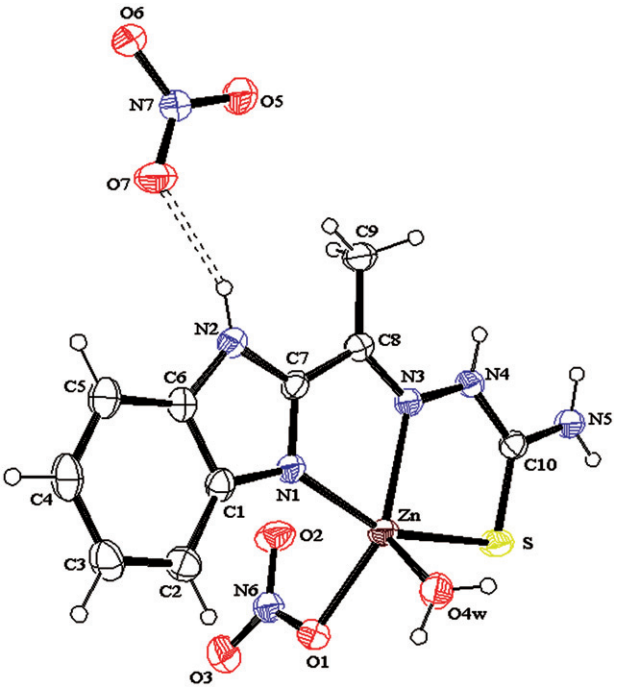

Figure 1. The molecular structure and atomic labeling scheme. Hydrogen bond is indicated by dashed lines. Displacement ellipsoids are drawn at the 40% probability level.

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to the dilutions under sterile conditions. The last row, which contained only the dilutions of the test samples without microorganism, was used as negative control. To eliminate solvent effects DMSO dilutions were considered as another control, separately. After incubation at 37 °C for 24 h the first well without turbidity was determined as the minimum inhibition concentration (MIC, $mg\,mL^{-1}$). As an antibacterial standard, Chloramphenicol was used and, similarly, the antifungal agent Ketoconazole was used as a control for the yeast. Results are given as averages of three experiments. Strain sources and activity results are given in table 2.

3. Results and discussion

3.1. *Synthesis, spectral studies, thermogravimetric analysis (TGA) and XRD measurement*

Reaction of $Zn(NO_3)_2 \cdot 6H_2O$ with 1-(1H-benzoimidazol-2-yl)-ethanone thiosemicarbazone at 1 : 1 metal to ligand molar ratio led to $[Zn(NO_3)(H_2O)(C_{10}H_{11}N_5S)]NO_3$, characterized as a pale yellow product with satisfactory C, H, and N elemental analyses. The coordination of the azomethine nitrogen atom to the metal ion is suggested by shifting of the ν(C=N) band at 1615 cm^{-1} to lower wavenumbers as in previous work [20]. Coordination of the sulfur causes, in the IR spectrum of the complex, shift of the band at 832 cm^{-1} to lower a frequency value (in the free ligand at 846 cm^{-1}) [20].

Table 2. Antimicrobial activity result (MIC, mg mL^{-1}) of the Zn complex.

Microorganisms	Strains	1	ST
Bacillus cereus, G(+)	NRRL B-3711	0.125	0.0039
Enterobacter aerogenes, G(−)	NRRL 3567	0.031	0.0039
Escherichia coli, G(−)	NRRL B-3008	0.25	0.0078
Pseudomonas aeruginosa, G(−)	ATCC 27853	0.5	0.125
Salmonella typhimurium, G(−)	ATCC 13311	0.062	0.0156
Staphylococcus aureus, G(+)	ATCC 6538	0.062	0.0019
Staphylococcus aureus (MRSA), G(+)	Clin. isol.	0.062	0.031
Candida albicans, yeast	ATCC 90028	2>	0.125*

ST: Chloramphenicol, antibacterial agent; * Ketoconazole, antifungal agent; MRSA: methycilline resistant; *S. aureus* Clin. isol.: clinical isolate from ESOGU

Medium bands at 1319 and 660 cm^{-1} are due to monodentate nitrate. In addition, small bands are present at 1046 and 805 cm^{-1} which can be attributed to the presence of non-coordinated nitrate. The observed values are in agreement with previous work [21].

The N(2)H at 12.60 ppm and N(4)H at 11.80 ppm (in the free ligand at 12.84 ppm and at 10.80 ppm, respectively) in the ^{1}H NMR spectra of the complex are due to the neutral ligand coordinating to zinc in a N_2S tridentate fashion.

The TGA profile of the complex showed that it is stable from ambient temperature to 443 °K. After that, the decomposition was observed in one stage. At 448 °K, the weight loss was *ca.* 4%, indicating loss from the crystal of one water. Finally, zinc oxide as the residue was observed around 473 K, after weight loss was ca. 80%.

Theoretical and experimental XRD patterns of sample match each other proving that the powder sample used and the crystal structure are of same material.

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3.2. *Crystal and molecular structure of* $[Zn(NO_3)(H_2O)(C_{10}H_{11}N_5S)]NO_3$

The structure of $[Zn(NO_3)(H_2O)(C_{10}H_{11}N_5S)]NO_3$ is shown in figure 1 and selected bond distances and angles are given in table 3. The coordination geometry about zinc is described as a distorted square pyramid. Estimating the structural index τ, which represents the relative amount of trigonality [square pyramid, $\tau = 0$; trigonal bipyramid, $\tau = 1$; $\tau = (\beta - \alpha)/60^\circ$ with α and β the two largest angles around the central atom] [22], N(1)–Zn–S β and N(3)–Zn–O(1) as α, $\tau = 0.16$, indicating that the arrangement about Zn^{II} is best described as a distorted square pyramid [$\alpha = 145.36(8)$ and $\beta = 155.13(6)^\circ$].

The Zn–O_{nitro} and Zn–OH_2 distances of $[Zn(NO_3)(H_2O)(C_{10}H_{11}N_5S)]NO_3$, which are slightly different, are the similar to five-coordinate bond distances [23]. Interatomic Zn–N and Zn–S bond distances [2.067(2), 2.190(3) and 2.3865(9) Å] are in agreement with other Zn–N and Zn–S bond distances reported in the literature [24, 25]. The coordination geometry is characterized by an $N(1)_{axial}$–Zn–S_{axial} angle of 155.13(6)° and $N(3)_{equatorial}$–Zn–$O(4w)_{equatorial}$ and $N(3)_{equatorial}$–Zn–$O(1)_{equatorial}$ angles ranging from 119.79(10) to 145.36(8)°. The axial distances are 2.3865(9) and 2.067(2) Å for Zn–S and Zn–N(1) and can be considered normal [26]. The equatorial bond distances of 2.0942(19) (Zn–O(1)), 2.067(2) (Zn–O4w) and 2.190(2) Å (Zn–N(3)) are normal for a square pyramid [26]. The two rings consisting of (Zn, N1, C7, C8, N3) and (Zn, S, C10, N4, N3) are planar. The dihedral angles between the planes of the two rings are 1.14(14)°. The Zn lies 0.007(8) and 0.0011(18) Å out of the (Zn, N1, C7, C8, N3) and

Table 3. Selected bond distances (Å) and angles (°) for the complex.

Zn–O1	2.0942(19)	S–C10	1.692(3)
Zn–S	2.3865(9)	N1–C1	1.386(3)
Zn–O4w	2.067(2)	N3–C8	1.285(3)
Zn–N1	2.067(2)	N3–N4	1.358(3)
Zn–N3	2.190(2)	N2–C6	1.380(3)
O1–N6	1.291(3)	N5–C10	1.317(3)
O4W–Zn–N1	94.52(9)	N3–Zn–S	79.33(6)
O4W–Zn–O1	94.46(9)	C10–S–Zn	99.99(9)
N1–Zn–O1	97.94(8)	N6–O1–Zn	105.33(15)
O4W–Zn–N3	119.79(10)	C7–N1–Zn	114.20(16)
N1–Zn–N3	75.83(8)	C1–N1–Zn1	39.76(17)
O1–Zn–N3	145.36(8)	C8–N3–Zn	117.52(16)
O4W–Zn–S	98.72(7)	N4–N3–Zn	119.71(15)
N1–Zn–S	155.13(6)	N5–C10–S	121.3(2)
O1–Zn–S	101.88(6)	N4–C10–S	123.16(19)

(Zn, S, C10, N4, N3) planes, respectively. There is an intermolecular N–H$\cdots$O hydrogen bond between N2 and O7 nitrate atoms. The parameters for the hydrogen bonding interaction (figure 1) in the molecule are as follows: H(2′)$\cdots$O(2) 2.003 Å, N(2)$\cdots$O(7) 2.809(3) Å, N(2)–H(2′)$\cdots$O(7) 155.55°.

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3.3. *Antimicrobial activity*

A random selection of human pathogenic Gram positive G(+), Gram negative G(−) bacteria and the yeast *Candida albicans* were treated with the Zn complex using a broth microdilution assay. The results showed that the G(−) *Enterobacter aerogenes* was the most susceptible bacteria against the tested complex with an inhibitory concentration of of $0.031\,mg\,mL^{-1}$. Good inhibitory effects against the G(+) the methycilline resistant *Staphylococcus aureus* (MRSA) and *S. aureus* (for both MIC $= 0.062\,mg\,mL^{-1}$) were observed. It is important to highlight that MRSA infections are becoming a major threat in hospitals, infants and elderly persons [27]. The same range of inhibition was also observed against the food pathogen *Salmonella typhimurium*. The yeast *Candida albicans* was rather resistant towards the complex when compared with Ketoconazole, as in table 2.

In previous work of Kasuga *et al*. [28], the antimicrobial activity of various Zn complexes showed inhibitory activities against a wide spectrum of microorganisms such as *E. coli*, *Bacillus subtilis*, *S. aureus*, *P. aeruginosa*, *C. albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger* and *Penicillium citrinum*. The relationship between the molecular structures was determined by single-crystal X-ray analysis and the observed antimicrobial activities suggested that zinc(II) complexes which could form intermolecular hydrogen bonding with a counter anion or hydrated water molecules showed modest to effective antimicrobial activities [28].

4. Conclusions

In this article, we prepare a new complex with a pentacoordinated environment of Zn(II) in a distorted square pyramid. Elemental analyses and all measurements show good agreement with the structure. The antimicrobial evaluation results suggest that

this complex is active on bacteria. It is worthwhile to evaluate the biological and pharmacological activities of further Zn complexes, because Zn(II) has a key role in many biological processes.

Supplementary material

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC, No. 664414 for compound $[Zn(NO_3)(H_2O)(C_{10}H_{11}N_5S)]NO_3$. Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-1223-336-033; E-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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