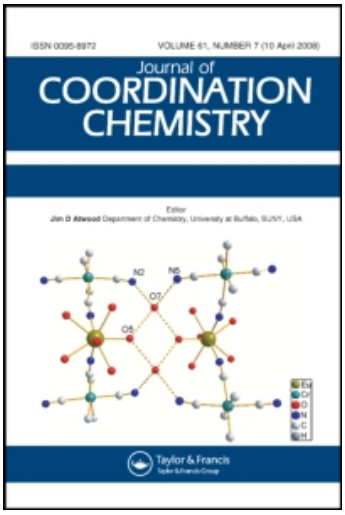

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Synthesis, characterization, and biological activity of a Schiff-base Zn(II) complex

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Synthesis, characterization, and biological activity of a Schiff-base Zn(II) complex

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Preparation and crystal structure of {4-chloro-2-[(2-morpholinoethylimino)methyl]phenolato} methanolchlorozinc(II) are reported. The X-ray structure reveals highly distorted square pyramidal geometry around zinc, binding to one phenolate O and two imine N atoms of the Schiff base, one methanol and one chloride. The complex and its ligand were tested *in vitro* for antibacterial and cytotoxic activity with a wide range of bactericidal activity and significant cytotoxic activity.

Keywords: Shiff base; Zn(II) complex; Crystal structure; Antibacterial; Cytotoxic

1. Introduction

Biology and structure–activity relationships (SAR) of Schiff-base compounds are studied due to their antitumor, antimicrobial and antiviral activities [1–4]. Schiff bases derived from substituted salicylaldehydes and amides show a variety of biological activities [5, 6]. Schiff bases play important roles in coordination chemistry related to catalysis and enzymatic reactions, magnetism and molecular architecture [7, 8], and also exhibit biological activities such as antimicrobial [9, 10] and cytotoxic activities [10, 11]. Zinc complexes have been shown to be active as antitumor, anti-HIV and antimicrobial agents [12–15]. We synthesized a new Zn(II) complex from a Schiff-base ligand condensed by 5-chlorosalicylaldehyde and 2-morpholinoethanamine. The antibacterial activities against *Bacillus subtilis* (Gram-positive), *Staphylococcus aureus* (Gram-positive), *Escherichia coli* (Gram-negative), *Pseudomonas fluorescence* (Gram-negative), and their cytotoxic activities against three tumor cell lines: KB, K562, and Hep-G2 were evaluated.

2. Experimental

2.1. *Chemistry*

(*E*)-4-*chloro*-2-[(2*morpholinoethylimino*)*methyl*]*phenol* was condensed from 5-chlorosalicylaldehyde and 2-morpholinoethanamine. Reaction of the Schiff base with $ZnCl_2$ in methanol led to formation of a new mononuclear Zn(II) complex (scheme 1).

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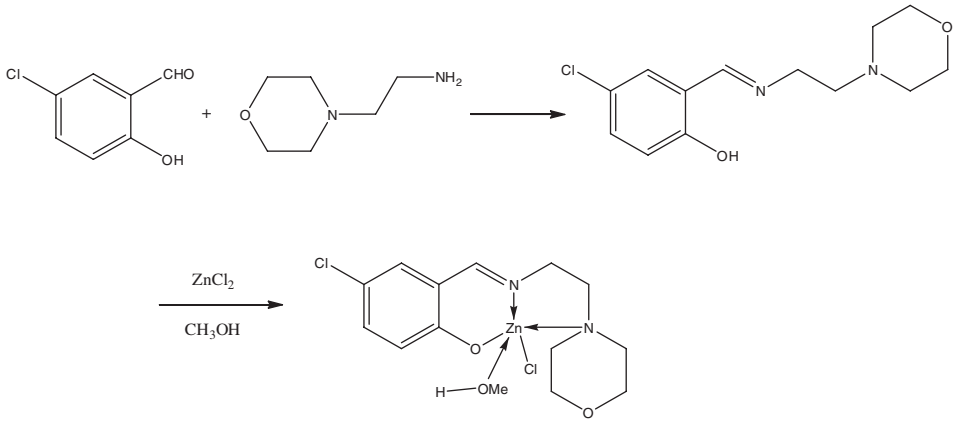

Scheme 1. Synthesis of Schiff base and complex.

All chemicals were of reagent grade and used as received. ^{1}H-NMR spectra were recorded on a Bruker DRX 500 model spectrometer in DMSO-d_6. Chemical shifts (δ) for ^{1}H-NMR spectra were reported in parts per million to residual solvent protons. ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument. Infrared spectra were recorded on a Nexus 870 FT-IR spectrometer in KBr disks at room temperature.

2.1.1. Preparation of the Schiff-base ligand. 156.5 mg (1.0 mmol) 5-chlorosalicylaldehyde and 130.1 mg (1.0 mmol) 2-morpholinoethanamine were dissolved in 10 mL methanol and stirred at room temperature for 30 min to give a clear solution. After standing for approximately 3 d, the precipitate was separated by filtration, washed with methanol and recrystallized from methanol to give the Schiff base (*E*)-4-*chloro*-2-[(2-*morpholinoethylimino*)*methyl*]*phenol*. Yield: 84%. ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 2.42 (m, 4H); 2.59 (t, $J = 6.4$ Hz, 2H); 3.55 (m, 4H); 3.72 (t, $J = 6.4$ Hz, 2H); 6.88 (d, $J = 8.9$ Hz, 1H); 7.33 (dd, $J = 8.9$ and 2.4 Hz, 1H); 7.53 (d, $J = 2.4$ Hz, 1H); 8.54 (s, 1H); 13.70 (s, 1H). ESI-MS: 267.2 ($C_{13}H_{16}ClN_2O_2^-$, $[M-H]^-$). Anal. Calcd for $C_{13}H_{17}ClN_2O_2$: C, 58.10%; H, 6.38%; N, 10.42%. Found: C, 58.24%; H, 6.36%; N, 10.43%.

2.1.2. Preparation of the Zn(II) complex. To a MeOH (10 mL) solution of $ZnCl_2$ (13.6 mg, 0.1 mmol) was added a MeOH solution (10 mL) of (*E*)-4-*chloro*-2-[(2*morpholinoethylimino*)*methyl*]*phenol* (26.8 mg, 0.1 mmol) with stirring. The mixture was stirred for 1 h at room temperature and then filtered. Upon keeping the filtrate in air for 10 days, colorless block-shaped crystals of the Zn(II) complex, suitable for X-ray crystal determination, formed on slow evaporation of the solvent. The crystals were isolated, washed three times with MeOH and dried in a vacuum desiccator containing anhydrous $CaCl_2$. Yield: 68%. Anal. Calcd for $C_{14}H_{20}Cl_2N_2O_3Zn$: C, 41.97%; H, 5.03%; N, 6.99%. Found: C, 41.72%; H, 4.96%; N, 7.06%. UV (nm): 387, 228. IR (KBr, cm^{-1}): 2958.4, 1635.5, 1523.9, 1465.7, 1388.6, 1320.9, 1178.0, 1116.4, 1058.9, 941.5, 828.4, 760.5.

Table 1. Crystallographic data for the complex.

Complex	Zn(II) complex
Formula	$C_{14}H_{20}Cl_2N_2O_3Zn$
Formula weight	400.59
Crystal shape/color	Block/colorless
Crystal size (mm^3)	$0.30 \times 0.20 \times 0.10$
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimension (Å, °)	
a	7.2500(14)
b	24.755(5)
c	9.6660(19)
α	90
β	101.63(3)
γ	90
V (Å^3)	1699.2(6)
Z	4
D_c (g cm^{-3})	1.566
μ (mm^{-1})	1.772
$F(000)$	824
T (K)	293(2)
θ range (°)	2.30/25.96
Index ranges	$0 \le h \le 8$, $0 \le k \le 30$, $-11 \le l \le 11$
Reflections collected/unique	3586/3322 [R(int) = 0.0729]
Data/restraints/parameters	3322/0/204
Max. and min. transmission	0.8427 and 0.6185
Goodness-of-fit on F^2	1.034
Final R indices [$I \ge 2\sigma(I)$][a]	$R_1 = 0.0517$, $\omega R_2 = 0.0933$
R indices (all data)[a]	$R_1 = 0.0936$, $\omega R_2 = 0.1097$
Largest difference peak and hole (e Å^{-3})	0.644 and −0.418

[a] $R_1 = \Sigma \| F_o | - | F_c \| / \Sigma | F_o |$, $wR_2 = [\Sigma w(F_o^2 - F_c^2)^2 / \Sigma w(F_o^2)^2]^{1/2}$.

Table 2. Selected bond lengths (Å) and angles (°) of the Zn(II) complex.

Cl(2)–Zn(1)	2.2182(13)	N(1)–Zn(1)	2.021(3)
N(2)–Zn(1)	2.517(4)	O(1)–Zn(1)	2.070(3)
O(3)–Zn(1)	2.029(3)		
N(1)–Zn(1)–O(3)	114.55(13)	N(1)–Zn(1)–O(1)	89.50(13)
O(3)–Zn(1)–O(1)	89.74(13)	N(1)–Zn(1)–Cl(2)	130.01(11)
O(3)–Zn(1)–Cl(2)	114.71(10)	O(1)–Zn(1)–Cl(2)	98.69(10)
N(1)–Zn(1)–N(2)	76.88(13)	O(3)–Zn(1)–N(2)	91.83(13)
O(1)–Zn(1)–N(2)	165.66(12)	Cl(2)–Zn(1)–N(2)	93.59(10)

2.2. *Crystal structure determination and refinement*

Crystal data, data collection and refinement parameters for Zn(II) complex are listed in table 1. Data were obtained on a Nonius CAD4 diffractometer equipped with graphite-monochromated Mo-Kα ($\lambda = 0.71073$ Å) radiation. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares using SHELX-97 [16]. All non-hydrogen atoms were refined anisotropically. All hydrogens were placed in calculated positions, assigned fixed isotropic thermal parameters at 1.2 times the equivalent isotropic U of the atoms to which they are attached and allowed to ride on their respective parent atoms. The contributions of these hydrogen atoms were included in the structure-factor calculations. Selected bond lengths and angles are listed in table 2.

2.3. *Antibacterial activity*

The antibacterial activities were tested against *B. subtilis*, *E. coli*, *P. fluorescence* and *S. aureus* using MH medium (Mueller–Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g, beef extract 1000 mL). The MICs (minimum inhibitory concentrations) of the test compounds were determined by a colorimetric method using the dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] [17]. A stock solution of the synthesized compound (50 μg mL^{-1}) in DMSO was prepared and quantities of the test compounds were incorporated in specified quantity of sterilized liquid MH medium. A specified quantity of the medium containing the compound was poured into microtitration plates. A suspension of the microorganism was prepared to contain approximately 10^5 cfu mL^{-1} and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37°C for 24 h. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (phosphate buffered saline 0.01 mol L^{-1}, pH 7.4: $Na_2HPO_4 \cdot 12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed and 100 μL of isopropanol containing 5% 1 mol L^{-1} HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm. The observed MIC values are presented in table 3.

2.4. *Cytotoxicity*

Cytotoxicities of the compounds against KB, K562 and Hep-G2 cells were evaluated as described elsewhere [18] with some modifications. Briefly, target tumor cells were grown to log phase in RPMI 1640 medium supplemented with 10% fetal bovine serum. After diluting to 2×10^4 cells mL^{-1} with the complete medium, 100 μL of the obtained

Table 3. MICs (minimum inhibitory concentrations) of the compounds.

	MICs (μg mL^{-1})			
	Gram-positive		Gram-negative	
Compounds	*B. subtilis*	*S. aureus*	*E. coli*	*P. fluorescence*
Ligand	>100	>100	12.5	12.5
Zn(II) complex	6.25	12.5	3.13	3.13
Penicillin	0.78	3.13	>100	>100
Kanamycin	0.39	1.56	6.25	6.25

Table 4. Cytotoxic activities of the compounds.

	IC_{50}/μM		
Compounds	K562	KB	Hep-G2
Ligand	77 ± 2	83 ± 2	69 ± 3
Zn(II) complex	22 ± 1	11.5 ± 0.7	9.8 ± 0.3
5-Fluorouracil	13.7 ± 0.4	13.4 ± 0.2	14.5 ± 0.5

cell suspension was added to each well of 96-well culture plates. Subsequent incubation was permitted at 37°C, 5% CO_2 atmosphere for 24 h before cytotoxicity assessment. Tested samples at pre-set concentrations were added to 6 wells with 5-fluorouracil co-assayed as a positive reference. After 48 h exposure period, 40 μL of PBS containing 2.5 mg mL^{-1} of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] was added to each well. Four hours later, 100 μL extraction solution (10% SDS–5% isobutyl alcohol–0.010 M HCl) was added. After an overnight incubation at 37°C;, the optical density was measured at a wavelength of 570 nm on an ELISA microplate reader. In all experiments three replicate wells were used for each drug concentration. Each assay was carried out at least three times. The results are summarized in table 4.

3. Results and discussion

Figure 1 gives a perspective view of the Zn(II) complex with the atom labeling system. X-ray analysis reveals the presence of a methanol in the metal coordination sphere. The geometry around Zn(II) can be described as a highly distorted square pyramid. Zn(II) is coordinated to one phenolate O and two imine N atoms of Schiff-base ligand, one methanol and one chloride. The Zn(1)–N(2) (2.517(4) Å) bond length is longer than the other Zn–N and Zn–O bonds (2.021(3) to 2.070(3) Å), which are in the normal range [19]. The angles subtended at Zn(II) range from 76.88(13)° to 165.66(12)°. Figure 2 gives the packing structure of the Zn(II) complex along the *a*-axis.

The Schiff base and its Zn(II) complex were screened for antibacterial activity against two Gram-positive bacterial strains (*B. subtilis* and *S. aureus*) and two Gram-negative bacterial strains (*E. coli* and *P. fluorescence*) by the MTT method. The MICs of the compounds against these bacteria are presented in table 3. The antibiotics kanamycin and penicillin were included as references. The Schiff base showed significant activity against two Gram-negative bacterial strains with MIC of 12.5 μg mL^{-1} but was inactive against two Gram-positive bacterial strains. The Zn(II) complex showed a wide range of bactericidal activities against the Gram-positive and Gram-negative bacteria, more potent than, or similar with, commercial antibiotics (kanamycin and penicillin).

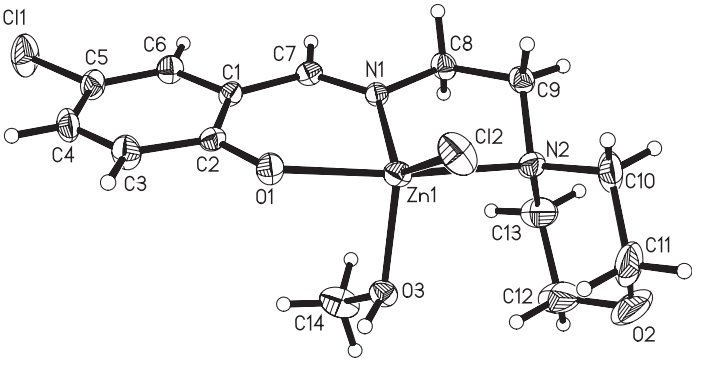

Figure 1. Crystal structure of the Zn(II) complex showing 30% probability displacement ellipsoids (arbitrary spheres for the H atoms).

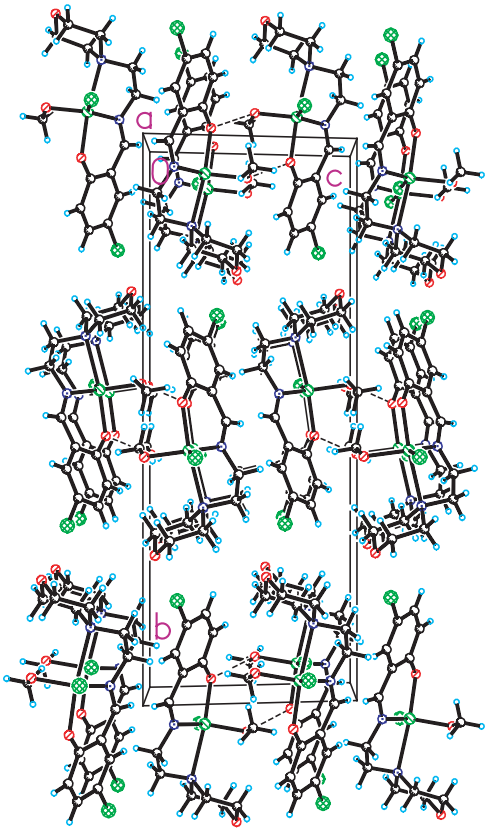

Figure 2. The packing structure of the Zn(II) complex along the *a*-axis.

The cytotoxic activities of the synthesized compounds against K562, KB and Hep-G2 summarized in table 4 indicate that the Zn(II) complex exhibits significant activity, similar to 5-fluorouracil, while the ligand exhibits low activity against the three tumor cell lines.

Acknowledgements

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