Synthesis, Crystal Structure and Antitumor Study of a Cobalt(II) Complex of the 2-Acetylpyrazine Thiosemicarbazone

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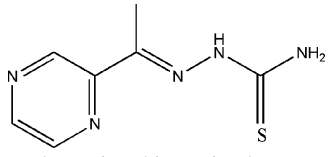
The title complex $[Co(C_7H_8N_5S)_2]\cdot 2H_2O$ has been synthesized and characterized by IR and UV spectral studies. The structure of the compound has been determined by single-crystal X-ray diffraction. The complex consists of discrete monomeric molecules with octahedrally hexacoordinate cobalt(II) ions, where two acetylpyrazine thiosemicarbazones act as NNS tridentate ligands coordinated to the central cobalt atom *via* the pyrazine nitrogen, azomethine nitrogen and sulfur atoms. Hydrogen bonds link the different components to stabilize the crystal structure. The antitumor activity of the title complex was tested against A549 lung cancer cell line. The complex exhibited lower antitumor activity, as compared to the free ligand.

Key words: Thiosemicarbazone Complex, Crystal Structure, Synthesis, Cytotoxic Activity

Introduction

Recently, there has been considerable interest in the chemistry of Schiff base compounds containing thiosemicarbazones and their metal complexes. Thiosemicarbazones are very promising molecules in coordination chemistry because of their pharmacological properties [1] which include notably antiparasital [2], antibacterial [3] and antitumor activities [4 – 5]. Many thiosemicarbazones, such as marboran or triapine, are already used in medical practice. The antitumor activity of some thiosemicarbazones increases by their ability to form chelates with specific metal ions [6]. It is noteworthy that thiosemicarbazones are versatile multifunctional chelating ligands that can coordinate as a neutral groups or in the deprotonated form, and also are flexible spacers with potential multiple binding sites that can be used to construct coordination polymers with multi-dimensional or supramolecular topological architectures [7]. The hydrogen atoms attached to the amino nitrogen atoms of the thiosemicarbazone moiety also have the ability to form donor hydrogen bonds from which small, simple fragments can be assembled into the desired cavities under favorable conditions, which is important in host-guest chemistry and has applications in chemistry, biology, and materials science [8].

Heterocyclic thiosemicarbazones are important because of their potentially beneficial biological properties [4]. A complex with pyrazineformamide N(4)-methylthiosemicarbazone has been reported [9]. We also described the synthesis and structure of transition metal [manganese(II), zinc(II), cadmium(II) and nickel(II)] complexes with the multidentate acetylpyrazine thiosemicarbazone ligand [10].



Scheme 1. Acetylpyrazine thiosemicarbazone, HL.

In the present paper, we report the synthesis, crystal structure and antitumor activity of a cobalt(II) complex derived from 2-acetylpyrazine thiosemicarbazone, HL (Scheme 1). The thiosemicarbazone ligand acts as a NNS tridentate ligand, coordinating through its pyrazine nitrogen, azomethine nitrogen and the sulfur atoms.

Experimental Section

General

Materials: All solvents and reagents are commercially available and were used without further purification. Acetylpyrazine thiosemicarbazone was prepared according to the literature method [11]. Instrumentation: Elemental analysis of C, H and N was performed with a Perkin-Elmer

Table 1. Summary of crystal data and refinement results for title complex.

Formula	$C_{14}H_{20}CoN_{10}O_2S_2$
M_r	483.45
Crystal size, mm^3	$0.2 \times 0.2 \times 0.2$
Crystal system	orthorhombic
Space group	*Pbcn*
a, Å	10.4618(9)
b, Å	22.6526(19)
c, Å	20.1794(17)
V, Å^3	4782.3(7)
Z	8
D_{calcd}, g cm^{-3}	1.343
$\mu(MoK_\alpha)$, cm^{-1}	0.921
θ Range for data collection, deg	2.1 – 25.0
$F(000)$, e	1992
hkl Range	$-10 \le h \le 12$, $-26 \le k \le 26$, $-23 \le l \le 19$
Refl. measured	4155
Refl. unique	3070
R_{int}	0.043
Param. refined	290
$R1/wR2$ $[I \ge 2\sigma(I)]$	0.064/0.216
$R(F)/wR(F^2)$ (all reflexions)	0.081/0.230
GoF	1.071
$\Delta\rho_{fin}$ (max/min), e Å^{-3}	1.48/−0.44

240 analyzer. The infrared spectra were recorded from KBr discs with a Nicolet 170 FT infrared spectrophotometer. Electronic spectra were obtained with a Unican UV-500 spectrometer (ethanol as solvent) from 200 to 700 nm.

Synthesis

An ethanol solution containing $Co(NO_3)_2 \cdot 6H_2O$ (0.036 g, 0.125 mmol) was added dropwise with constant stirring and slow heating to 30 mL of a solution of acetylpyrazine thiosemicarbazone (0.049 g, 0.25 mmol) in the same solvent. The solution immediately turned deep red. After stirring for 0.5 h, the resultant solution was filtered. Deep-red crystals suitable for X-ray studies were obtained by slow evaporation of an ethanol solution. Yield: 65 % – $C_{14}H_{20}CoN_{10}O_2S_2$ (483.45): calcd. C 34.78, H 4.14, N 28.98; found C 34.72, H 4.18, N 28.99.

X-Ray crystallographic study

A red crystal with approximate dimensions of $0.2 \times 0.2 \times 0.2$ mm^3 was mounted on a glass fiber in a random orientation. Crystallographic data were collected with a Siemens SMART-CCD diffractometer with graphite-monochromated MoK_α radiation (λ = 0.71073 Å). A total of 22354 reflections was measured by ω scan technique at 293(2) K within $2.1 \le \theta \le 25.0°$, of which 4155 were independent with R_{int} = 0.0427, and 3070 were observed with $I \ge 2\sigma(I)$. The structure was solved by Direct Methods and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for all non-hydrogen atoms using SHELXTL [12]. The hydrogen atoms were added in idealized geometrical positions. Final R indices [$I \ge 2\sigma(I)$]: $R1$ = 0.064, $wR2$ = 0.216. Table 1 summarizes crystal and refinement data.

Table 2. Selected bond lengths (Å) and angles (deg) of the title complex.

Co(1)–N(3)	1.880(2)	Co(1)–N(8)	1.885(2)
Co(1)–N(5)	1.956(3)	Co(1)–N(9)	1.951(2)
Co(1)–S(1)	2.222(1)	Co(1)–S(2)	2.217(1)
S(1)–C(1)	1.743(3)	S(2)–C(8)	1.738(3)
N(2)–C(1)	1.316(4)	N(7)–C(8)	1.322(4)
N(2)–N(3)	1.377(3)	N(7)–N(8)	1.371(4)
N(3)–C(3)	1.299(4)	N(8)–C(10)	1.311(4)
N(5)–C(4)	1.360(4)	N(9)–C(11)	1.347(4)
N(3)–Co(1)–N(8)	178.8(1)	N(5)–Co(1)–S(1)	169.3(1)
N(9)–Co(1)–S(2)	169.2(1)	N(3)–Co(1)–S(2)	95.0(1)
N(3)–Co(1)–N(9)	95.9(1)	N(5)–Co(1)–N(9)	90.8(1)
N(5)–Co(1)–S(2)	90.7(1)	S(1)–Co(1)–S(2)	90.9(1)
S(1)–Co(1)–N(9)	89.6(1)	N(8)–Co(1)–N(9)	83.0(1)
N(8)–Co(1)–S(2)	86.2(1)	N(3)–Co(1)–N(5)	82.6(1)
N(8)–Co(1)–N(5)	97.1(1)	N(8)–Co(1)–S(1)	93.6(1)
N(3)–Co(1)–S(1)	86.7(1)		

CCDC number 658007 contains the supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

In vitro cytotoxicity study

A549, a human lung cancer cell line (purchased from the Institute of Biochemistry and Cell Biology, SIBS, CAS) was cultured in *RPMI-1640* medium supplemented with 10 % FBS, 100 U/mL of penicillin, 100 μg mL^{-1} of streptomycin at 37 °C in humid air atmosphere of 5 % CO_2. Cell cytotoxicity was assessed by the MTT assay. Briefly, cells were placed into a 96-well-plate (5×10^3 cells/well). The next day the compound at various concentration diluted in culture medium was added to the wells (200 μL/well). 48 h later 20 μL of MTT (0.5 mg mL^{-1} MTT in PBS) was added and cells were incubated for a further 4 h. 200 μL of DMSO were added to each culture to dissolve the reduced MTT crystals. The MTT-formazan product dissolved in DMSO was estimated by measuring absorbance at 570 nm with a micro plate reader. Then the inhibitory percentage of each compound at various concentrations was calculated, and the IC_{50} value was determined.

Results and Discussion

X-Ray crystal structure

The molecular structure of the complex along with the atomic numbering scheme is shown in Fig. 1. Selected bond lengths and angles are listed in Table 2, hydrogen bond lengths and angles in Table 3.

Table 3. Hydrogen bond lengths (Å) and bond angles (deg).

D–H···A	*d*(H···A)	*d*(D···A)	∠(DHA)
N(1)–H(1A)···S(2)	2.84	3.477(3)	132.5
N(1)–H(1B)···N(4)	2.10	2.960(4)	177.8
N(6)–H(6B)···N(10)	2.24	2.953(4)	141.0
N(6)–H(6C)···O(2W)	2.00	2.862(8)	177.4

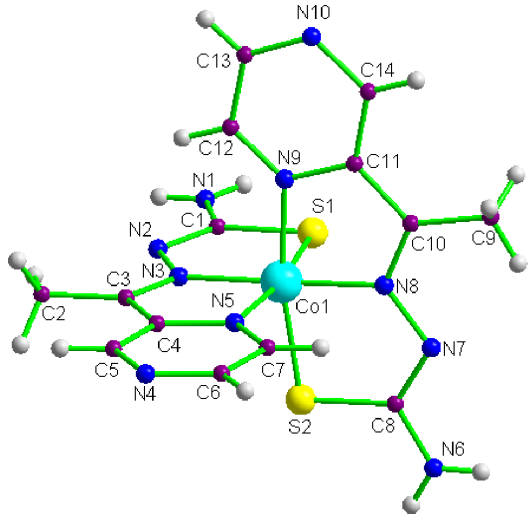


Fig. 1. Molecular structure of the title compound.

The crystal structure of $[Co(C_7H_8N_5S)_2]\cdot 2H_2O$ contains discrete $[Co(C_7H_8N_5S)_2]$ entities and two disordered water solvate molecules. The cobalt(II) ions are hexa-coordinated with four nitrogen and two sulfur atoms from two identical N_2S tridentate L^- ligands, which are similar to those in the Mn(II) complex with the same ligand [10]. One sulfur atom, one imine and one pyrazine nitrogen atom from one ligand and one imine nitrogen atom from another ligand occupy the basal positions, the two remaining axial positions in the octahedral geometry are occupied by one sulfur atom and one pyrazine nitrogen atom from different ligands. The pseudo-macrocyclic coordination mode of each ligand affords two five-membered chelate rings, which are nearly planar. The dihedral angles between the mean planes are 1.4° and 0.7°, respectively.

The thiosemicarbazone ligands can be considered planar with mean deviations from the best plane of 0.012 and 0.009 Å, respectively. The C1–S1 and C8–S2 bond lengths of 1.743(3) and 1.738(3) Å, respectively, are within the normal range of C–S single bonds, indicating that the thiosemicarbazone moieties adopt the thiol tautomeric form [13]. The C–N and N–N bond lengths in L^- are intermediate between formal single and double bonds, pointing to an extensive electron delocatization over the entire molecular skeleton. Furthermore, the bond angles in the acetylpyrazine thiosemicarbazone ligand of approximately $120 \pm 5°$ are also compatible with the electron delocalization [14]. The two pyrazine rings (mean plane deviations of 0.0036 and 0.0043 Å) form a dihedral angle of 89.3°.

The distances of Co1–S1 (2.222(1) Å), Co1–S2 (2.217(1) Å), Co1–N3 (1.880 (2) Å) and Co1–N8 (1.885(2) Å) are similar to those found in cobalt complexes with methylated isatin and ferrocene-containing thiosemicarbazones [15 – 16].

The existence of intermolecular hydrogen bonds involving the coordinated sulfur (S2) atom, the oxygen atoms of the water molecules, the uncoordinated nitrogen atoms of the pyrazine ring and terminal amine group assembles the neutral complex into a supermolecule with a 2D layer structure along the *b* axis. The uncoordinated pyrazine nitrogen atoms act as hydrogen bond acceptors while the uncoordinated terminal amino nitrogen atoms act as donors. The separations for N1···N4 and N6···N10 are 2.960(4) and 2.953(4) Å with the N–H···N angles at 177.8° and 141.0°, respectively. Additionally, the intermolecular distances N1···S2 and N6···O2W are 3.477(3) and 2.862(8) Å with angles N1–H1A···S2 and N6–H6C···O2W at 132.5° and 177.4°, respectively. The molecular packing by hydrogen bonds gives well defined channels running down the *b* axis in which the lattice water molecules O1W are located.

IR spectra

The infrared spectral bands most useful for determining the mode of coordination of the ligands are the ν(C=N) and ν(C=S) vibrations. The IR spectra of the free ligand and the title complex all have three bands around 3118, 3283 and 3410 cm^{-1}, which can be assigned to the ν(N–H) vibrations, indicating that the amino nitrogen atoms do not participate in coordination. The ν(C=N) bands of the thiosemicarbazone and of the complex are found at 1595 and 1558 cm^{-1}, respectively. The decrease in frequency of this band in the spectra of the complex is an evidence for the coordination *via* the azomethine nitrogen atom [17]. The band at 850 cm^{-1} observed for the uncomplexed thiosemicarbazone can be attributed to the ν(C=S) vibration. This band is shifted to lower en-

ergies (825 cm^{-1}) in the complex, indicating the coordination of the thiolato sulfur atom.

UV spectra

The electronic absorption spectra of the acetylpyrazine thiosemicarbazone ligand and the Co(II) complex were recorded in ethanol. The HL ligand shows three bands at 204, 248 and 324 nm, which are assigned to pyrazine (π–π^*), thiosemicarbazone (π–π^*) and (n–π^*), respectively [18]. Similarly, the electronic spectrum of the title complex also features three bands. The band observed at *ca.* 208 nm can be assigned to the π–π^* transitions of pyrazine. Commonly n–π^* transitions involving N and S atoms occur at a lower energy than π–π^* transitions [19]. Additional bands present at 240 and 328 nm are thus assignable to thiosemicarbazone π–π^* and n–π^* transitions, respectively.

In vitro cytotoxic activity

Taking into account that thiosemicarbazone molecules show cytotoxic activity [20] and that transition metal compounds may have antitumor properties [21], we have tested the ability of the Co(II) complex to inhibit tumor cell growth. In our experiment, IC_{50} values (compound concentration that produces 50 % of cell death) in micro molar units were calculated for the free ligand and the complex against lung cancer A549 cell lines. It is worth noting that the free ligand showed a lower IC_{50} value (18.9 μM) than the cobalt complex (265.3 μM). In our earlier studies, it also was found that the cobalt complex of another thiosemicarbazone ligand exhibited poorer antitumor activity compared to the free ligand against the same cell line of human lung cancer. The lack of biological activity of the cobalt compound could be related to the presence of a complete octahedral coordination [22 – 24].

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