Synthesis, Crystal Structure and Antitumor Study of an Iron(III) Complex of 2-Acetylpyrazine *N*(4)-Methylthiosemicarbazone

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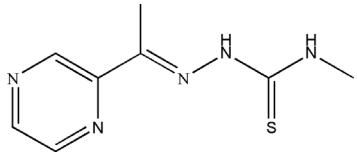
The title complex $[Fe(C_8H_{10}N_5S)_2]\cdot Cl$ has been synthesized from 2-acetylpyrazine *N*(4)-methylthiosemicarbazone and $FeCl_3\cdot 6H_2O$ and characterized by elemental analysis, IR spectroscopy and single-crystal X-ray diffraction. The complex consists of discrete monomeric molecules with octahedrally hexacoordinated iron(III) ions, in which two 2-acetylpyrazine 4-methylthiosemicarbazone units act as meridional NNS tridentate ligands coordinated to the central iron atom *via* the pyrazine nitrogen, azomethine nitrogen and sulfur atoms. Hydrogen bonds link the complex components to stabilize the crystal structure. The antitumor activity of the title complex was tested against K562 leucocythemia and BEL7402 liver cancer cell lines. The complex exhibits higher antitumor activity as compared to the free ligand.

Key words: Thiosemicarbazone Complex, Crystal Structure, Cytotoxic Activity

Introduction

Thiosemicarbazones have experienced long-standing applications in biology and medicine [1 – 2] because of their significant antifungal, antiprotozoal, antibacterial and anticancer activity [3 – 4] as well as in the catalysis of chemical and petrochemical processes [5 – 6]. Triapin has been tested in phase I trials for patients with advanced cancer [7]. The biological activity of certain thiosemicarbazones is due to their ability to form tridentate chelates with transition metal ions bonding through oxygen, nitrogen and sulfur atoms, O–N–S [8], or sulfur and two nitrogen atoms, S-N-N [9]. Firstly, lipophilicity, which controls the rate of entry into the cell, is modified by coordination. Also, the metal complex can be more active than the free ligand, and some side effects may decrease upon complexation. So thiosemicarbazones containing a pyridine ring give rise to NNS tridentate systems, and their metal complexes have stimulated widespread interest regarding the biological activities [10 – 12]. On the other hand, many studies have suggested that iron complexes have significantly greater antitumor activity than the free ligand [13 – 15]. The cytotoxicity and antitumor activity of thiosemicarbazone-iron(III) complexes may be related to the reaction of complexes with cell thiols or thiol-containing proteins [16]. In our previous studies we found that 2-acetylpyrazine thiosemicarbazone and its cobalt(II) complex exhibit antitumor activities against lung cancer A549 cell lines [17]. In addition, it was found that the presence of a bulky group at the terminal nitrogen N(4) considerably increases the activity [18].



Scheme 1. 2-Acetylpyrazine *N*(4)-methylthiosemicarbazone, HL.

Continuing with our research program concerning the biological properties of thiosemicarbazones [17, 19 – 20], we have widened the scope of investigations to 2-acetylpyrazine *N*(4)-methylthiosemicarbazone (HL, Scheme 1) with the final goal to develop new biologically active pharmaceuticals. Here, we report the synthesis, crystal structure and antitumor activity of its iron(III) complex. The title complex exhibits higher antitumor activity against K562 leucocythemia and BEL7402 liver cancer cell lines, as compared to the free ligand.

Experimental Section

General

Materials: All solvents and reagents are commercially available and were used without further purification. 2-Acetylpyrazine *N*(4)-methylthiosemicarbazone was prepared according to the literature method [21].

Instrumentation: Elemental analysis of C, H and N was performed with a Perkin-Elmer 240 analyzer. The infrared spectra were recorded from KBr discs with a Nicolet 170 FT infrared spectrophotometer.

Synthesis

An ethanol solution containing $FeCl_3 \cdot 6H_2O$ (0.034 g, 0.13 mmol) was added dropwise with constant stirring and slow heating to 30 mL of a solution of acetylpyrazine *N*(4)-methylthiosemicarbazone (0.052 g, 0.25 mmol) in the same solvent. After refluxed for 5 h, the resultant solution was filtered. Deep-red crystals of the title compound suitable for X-ray studies were obtained by slow evaporation of its ethanol solution.

Elemental analysis for $C_{16}H_{20}ClFeN_{10}S_2$: calcd. C 37.83, H 3.94, N 27.59; found C 37.58, H 4.01, N 27.66.

X-Ray crystallographic study

A red crystal with approximate dimensions of $0.20 \times 0.18 \times 0.16$ 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 11748 reflections was measured by ω-scan technique at 296(2) K within $2.03 \leq \theta \leq 25.49°$, of which 4128 were independent with R_{int} = 0.112, and 1760 were observed with $I \geq 2\sigma(I)$. The structure was solved by Direct Methods and refined by full-matrix least-squares calculations on F^2 with anisotropic displacement parameters for all non-hydrogen atoms using SHELXTL [22]. The hydrogen atoms were added in idealized geometrical positions. Final *R* indices [$I \geq 2\sigma(I)$]: $R1 = 0.047$, $wR2 = 0.078$. Table 1 summarizes crystal and refinement data.

CCDC number 693161 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

K562 (a human leucocythemia cancer cell line) and BEL7402 (a liver cancer cell line), purchased from the Institute of Biochemistry and Cell Biology, SIBS, CAS, were cultured in RPMI-1640 medium supplemented with 10 % FBS, 100 U mL^{-1} of penicillin, 100 μg (200 μL per well) 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 per well). The next day the compound diluted in culture medium at various concentrations was added (200 μL per well) to the wells. 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 was added to each culture to dissolve the 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.

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

Formula	$C_{16}H_{20}ClFeN_{10}S_2$
M_r	507.84
Crystal size, mm^3	$0.20 \times 0.18 \times 0.16$
Crystal system	monoclinic
Space group	$P2_1/n$
a, Å	11.362(1)
b, Å	13.502(2)
c, Å	14.921(2)
β, deg	104.450(2)
V, Å^3	2216.6(4)
Z	4
$D_{calcd.}$, g cm^{-3}	1.522
μ(MoK_α), cm^{-1}	1.015
θ range for data collection, deg	2.03 – 25.49
F(000), e	1044
hkl range	$-10 \leq h \leq 13$, $-16 \leq k \leq 14$, $-18 \leq l \leq 14$
Refl. measured	4128
Refl. unique	1760
Param. refined	275
R_{int}	0.112
$R1(F)/wR2(F^2)$ [$I \geq 2\sigma(I)$]	0.047/0.078
$R1(F)/wR2(F^2)$ (all refls.)	0.137/0.093
Gof (F^2)	0.677
$\Delta\rho_{fin}$ (max/min), e Å^{-3}	0.31/–0.41

Results and Discussion

IR spectra

The infrared spectral bands most useful for determining the mode of coordination of the ligands are the iminic ν(C=N) and ν(C=S) vibrations. The IR spectra of the free ligand and the title complex do not display ν(S–H) bonds at 2600 cm^{-1}, indicating that in the solid state both the free ligand and the title complex remain in the thione form [23]. The ν(N–H) band of the free ligand is found at 3307 cm^{-1}, but has disappeared in the spectrum of the title complex, as a result of ligand deprotonation. The ν(C=N) bands of the free ligand and the title complex are found at 1610 and

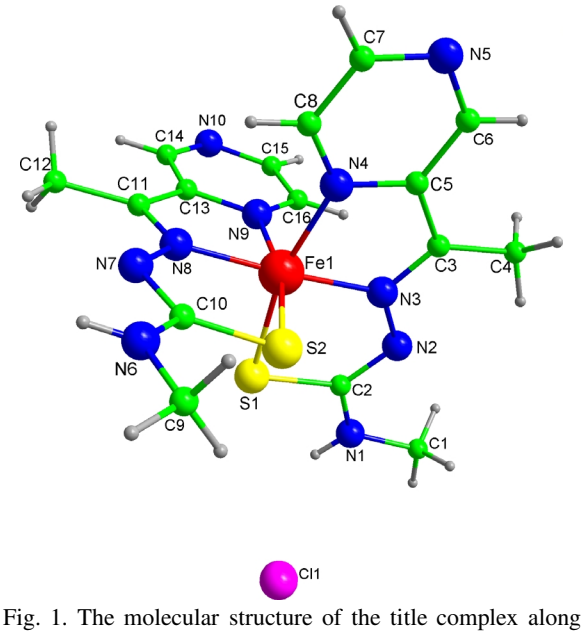


Fig. 1. The molecular structure of the title complex along with the numbering scheme.

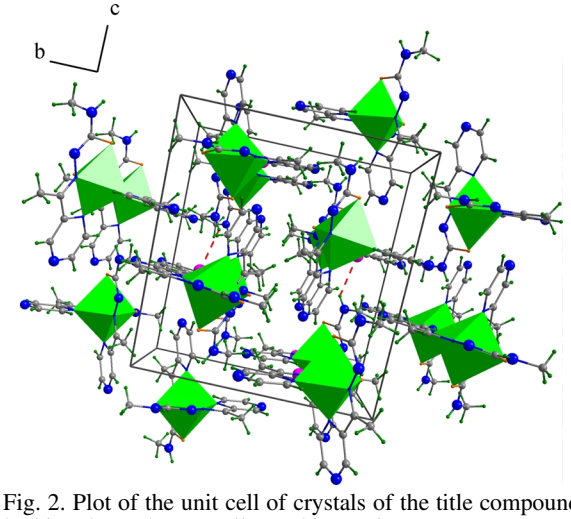


Fig. 2. Plot of the unit cell of crystals of the title compound looking down the crystallographic *a* axis.

1587 cm^{-1}, respectively. The decrease in frequency of this band in the spectrum of the title complex is an evidence for the coordination *via* the azomethine nitrogen atoms (N8 and N3; see Fig. 1) [24]. The band at 837 cm^{-1} observed for the free ligand can be attributed to the ν(C=S) vibration. This band is shifted to lower energies (829 cm^{-1}) in the title complex, indicating the coordination of the sulfur atom. These observations have been confirmed by X-ray single crystal structure analysis.

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

Fe(1)–N(8)	1.894(3)	Fe(1)–N(3)	1.909(4)
Fe(1)–N(9)	1.922(3)	Fe(1)–N(4)	1.941(4)
Fe(1)–S(2)	2.296(1)	Fe(1)–S(1)	2.297(2)
S(1)–C(2)	1.754(5)	S(2)–C(10)	1.697(5)
N(1)–C(2)	1.327(6)	N(1)–C(1)	1.453(5)
N(2)–C(2)	1.321(6)	N(2)–N(3)	1.369(5)
N(3)–C(3)	1.306(5)	N(6)–C(10)	1.321(5)
N(6)–C(9)	1.454(5)	N(7)–C(10)	1.359(5)
N(7)–N(8)	1.388(4)	N(8)–C(11)	1.306(5)
N(8)–Fe(1)–N(3)	174.9(2)	N(8)–Fe(1)–N(9)	81.1(2)
N(3)–Fe(1)–N(9)	94.1(2)	N(8)–Fe(1)–N(4)	97.4(2)
N(3)–Fe(1)–N(4)	81.1(2)	N(9)–Fe(1)–N(4)	94.2(2)
N(8)–Fe(1)–S(2)	85.2(1)	N(3)–Fe(1)–S(2)	99.6(1)
N(9)–Fe(1)–S(2)	166.3(1)	N(4)–Fe(1)–S(2)	88.5(1)
N(8)–Fe(1)–S(1)	97.0(1)	N(3)–Fe(1)–S(1)	84.5(1)
N(9)–Fe(1)–S(1)	88.9(1)	N(4)–Fe(1)–S(1)	165.5(1)
S(2)–Fe(1)–S(1)	91.9(1)		

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

D–H···A	*d*(H···A)	*d*(D···A)	∠(DHA)
N(6)–H(6B)···Cl(1)	2.38	3.177(4)	154.1
N(1)–H(1A)···Cl(l)	2.63	3.329(5)	138.6

X-Ray crystal structure

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

The crystal structure of the title complex contains discrete $[Fe(C_8H_{10}N_5S)_2]^+$ entities and one chloride anion. In the cation, the iron(III) ion is in a slightly distorted octahedral environment, where two acetylpyrazine *N*(4)-methylthiosemicarbazone units act as meridional NNS tridentate ligands coordinated to the central iron atom *via* the pyrazine nitrogen, azomethine nitrogen and sulfur atoms, which is similar to what is found in the analogous thiosemicarbazone compounds [25]. 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 chelate rings in the two ligands are 0.9° and 3.2°, respectively.

The two pyrazine rings (mean plane deviations of 0.003 and 0.006 Å) form a dihedral angle of 97.6°.

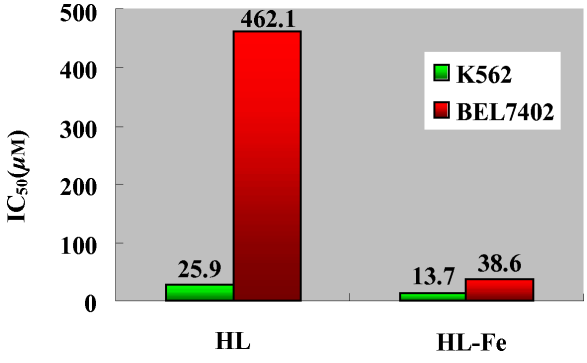


Fig. 3. The antitumor activities of the free ligand and the title complex against K562 leucocythemia cell lines and BEL7402 liver cancer cell lines.

The thiosemicarbazone ligands can be considered planar with mean deviations from the best plane of 0.055 and 0.032 Å, respectively. The C(2)–S(1) bond length of 1.754(5) Å and the C(10)–S(2) bond length of 1.697(5) Å agree well with those in related complexes, being intermediate between 1.82 Å for a C–S single and 1.56 Å for a C=S double bond [26], thus pointing to an extensive electron delocatization over the entire molecular skeleton [27]. The C(3)–N(3) and C(11)–N(8) bond lengths are 1.306(5) and 1.306(5) Å, respectively, which indicates that the linkages are all double bonds. The C(2)–N(2), C(2)–N(1), C(1)–N(1), C(10)–N(7), C(10)–N(6), C(9)–N(6), N(2)–N(3), and N(7)–N(8) bond lengths are all in the single bond range.

The title complex is entertaining a number of hydrogen bonds. They involve the uncoordinated nitrogen atoms N(6) and N(1), and the chlorine atom. The uncoordinated nitrogen atoms act as hydrogen bond donors while the chlorine atom acts as an acceptor. The separations for N(6)···Cl(1) and N(1)···Cl(1) are 3.177(4) and 3.329(5) Å with N–H···Cl angles at 154.1° and 138.6°, respectively.

In vitro cytotoxic activity

Taking into account that thiosemicarbazone molecules show cytotoxic activity [28], and their transition metal complexes may have enhanced antitumor properties [29], we have tested the ability of the free ligand and the title complex to inhibit tumor cell growth. In our experiments, IC_{50} values (compound concentration that produces 50 % of cell death) in micro-molar units were calculated for the free ligand and the title complex against two human cancer cell lines: K562 (leucocythemia cell line) and BEL7402 (liver cancer cell line). We found that both the free ligand and the title complex show significant antitumor activity against K562 leucocythemia cells, and the free ligand exhibits very poor cytotoxic activity against BEL7402 liver cancer cell lines (Fig. 3). It is worth noting that the title complex showed a lower IC_{50} value (13.7 μM for K562, 38.6 μM for BEL7402) than the free ligand (25.9 μM for K562, 462.1 μM for BEL7402). Obviously, coupling of 2-acetylpyrazine *N*(4)-methylthiosemicarbazone to iron(III) resulted in a higher antitumor activity than that of the free ligand, as it was found in other iron compounds [13 – 15]. This confirms the conclusion that the antitumor activities of a thiosemicarbazone can be increased by coordinating the ligand to metal cations [30]. In addition, both the free ligand and its iron(III) complex show higher antitumor activities against K562 leucocythemia cells than against BEL7402 liver cancer cells. These results indicate that the iron(III) title complex has the potential to be considered as an antitumor agent candidate for further stages of screening *in vitro* and/or *in vivo*.

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