Synthesis, Characterization, Crystal Structure, and Cytotoxicity of a 7-Coordinate Diorganotin(IV) Complex of 2-Acetylpyrazine N^4-Methylthiosemicarbazone

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The diorganotin(IV) complex $[Ph_2Sn(L)(CH_3COO)]$ (**1**), where HL = 2-acetylpyrazine N^4-methyl thiosemicarbazone, has been synthesized and characterized by elemental analysis, IR, UV/Vis and NMR spectroscopy, mass spectrometry, and single-crystal X-ray diffraction. Complex **1** contains mononuclear neutral molecules composed of one N_2S tridentate anionic thiosemicarbazone ligand, one acetato group, and one Ph_2Sn(IV) group with a seven-coordinated tin atom. *In vitro* biological studies have indicated that complex **1** shows effective cytotoxicity with IC_{50} = 5.4 μM against the K562 leukaemia cell line.

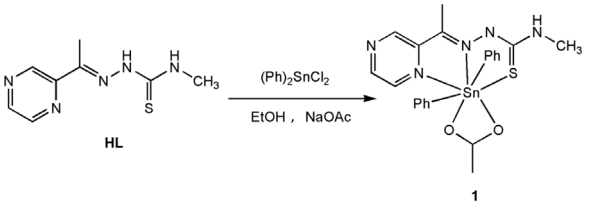
Key words: Thiosemicarbazone, Diorganotin(IV), Crystal Structure, Cytotoxic Activity

Introduction

Heterocyclic thiosemicarbazones and their metal complexes have received considerable attention in chemistry and biology, owing to their marked and variable biological properties [1 – 6]. The biological activities of thiosemicarbazones often depend on the parent aldehyde or ketone. On the other hand, the biological properties of metal thiosemicarbazones can be modified and often differ from those of either the ligands or the metal ions with changes in the metal ion coordination [7 – 9]. In some cases the highest *in vivo* activity is associated with a metal complex rather than the parent ligand, and some side effects may decrease upon complexation [10, 11].

Tin complexes are known for their multiple applications as antimicrobials and biocides [12]. Moreover, diorganotin(IV) compounds are known to exhibit important cytotoxic effects against tumor cell lines [13 – 15].

In recent years we have been working on the structural and biological properties of heterocyclic thiosemicarbazones and their metal complexes [16]. The results have revealed that thiosemicarbazones derived from 2-acetylpyrazine and their metal complexes show significant antitumor activities. After a careful literature search, we can affirm that 2-acetylpyrazine N^4-substituted thiosemicarbazones and their diorganotin(IV) complexes are scarce. Therefore, it seemed important for us to obtain their tin complexes as a strategy for the preparation of new drug candidates in which the metal and ligand could act synergistically.



Scheme 1. The reaction scheme for the synthesis of **1**.

From the present study, we describe the synthesis, characterization and cytotoxicity of the 7-coordinate diorganotin(IV) complex **1** with 2-acetylpyrazine N^4-methylthiosemicarbazone as ligand (Scheme 1).

Experimental Section

General

Materials: All solvents and reagents were commercially available and used without further purification. 2-acetylpyrazine N^4-methylthiosemicarbazone was prepared according to the literature method [17].

Instrumentation

Elemental analysis of C, H and N was performed on a Perkin-Elmer 240 analyzer. The infrared spectra were recorded from KBr discs on a Nicolet 170 FT infrared spectrophotometer. Electronic spectra were obtained with a Hitachi U4100 spectrometer. ^{1}H NMR spectra were recorded using a Bruker AV-400 spectrometer. The masss spectra were taken on an Esquire 3000 LC-MS spectrometer.

Synthesis

An ethanol solution containing Ph_2SnCl_2 (0.068 g, 0.2 mmol) was added dropwise to an ethanol solution (20 mL) of 2-acetylpyrazine N^4-methylthiosemicarbazone (0.042 g, 0.2 mmol) and NaOAc (0.016 g, 0.2 mmol). After refluxing for 2 h with stirring, the resulting mixture was filtered. Orange crystals suitable for X-ray studies were obtained by slow evaporation of an ethanol solution. – Elemental analysis for $C_{22}H_{23}SnN_5SO_2$: calcd. C 48.92, H 4.29, N 12.96; found C 49.02, H 4.64, N 12.78. – UV/Vis (C_2H_5OH): λ_{max} = 320 nm. – ^{1}H NMR($CDCl_3$, ppm): 9.21 (s, 1H, NH), 8.64 (s, 1H, Pz), 8.53 (d, J = 1.6 Hz, 1H, Pz), 8.50 (d, J = 2.4 Hz, 1H, Pz), 7.74 – 7.72 (m, 2H, Ph), 7.62 (t, J = 4 Hz, 4H, Ph), 7.46 – 7.44 (m, 4H, Ph), 3.29 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.09 (s 3H, CH_3COO^-). – MS ((+)-ESI): m/z = 481.4 $[(Ph)_2Sn(L)]^+$. The complex is soluble in CH_3OH, C_2H_5OH, DMF, and DMSO.

X-Ray crystallographic study

An orange crystal with approximate dimensions of $0.47 \times 0.21 \times 0.03$ mm^3 was mounted in random orientation on a glass fiber. Intensity data were collected at T = 296(2) K on a Siemens SMART-CCD diffractometer with graphite-monochromatized MoK_α radiation (λ = 0.71073 Å) using the programs SMART and SAINT [18]. The structure was solved by Direct Methods and refined by full-matrix least-squares based on F^2. All non-hydrogen atoms were refined anisotropically. The positions of hydrogen atoms were added in idealized geometrical positions. Details are given in Table 1.

CCDC 800548 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

Cytotoxicity assay

K562 leukaemia cells (purchased from Institute of Biochemistry and Cell Biology, SIBS, CAS) were placed into 96-well plates at a density of 1×10^4 cells per well and allowed to grow in a CO_2 incubator. After 24 h, the medium was removed and replaced by fresh medium containing the tested compounds which were dissolved in DMSO at 0.01 M and diluted to various concentrations with phosphate-buffered saline (PBS) before the experiment. The final concentration of DMSO was lower than 1 %. After 24 h incubation, cultures were incubated in 100 μL of a medium with 10 μL of a 5 mg mL^{-1} MTT solution for 4 h at 37 °C. The medium with MTT was removed, and 100 μL of DMSO was added to each well to dissolve the formazan. The absorbance at 570 nm was measured with a microplate reader (Bio-Tek ELX800, USA). 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 numbers pertinent to data collection and structure refinement for complex **1**.

Formula	$C_{22}H_{23}SnN_5SO_2$
M_r	540.200
Crystal size, mm^3	$0.47 \times 0.21 \times 0.03$
Crystal system	monoclinic
Space group	$C2/c$
a, Å	28.112(3)
b, Å	10.282(1)
c, Å	16.729(2)
V, Å^3	4772.9(9)
Z	8
D_{calcd}, g cm^{-3}	1.50
μ(MoK_α), cm^{-1}	1.2
θ range data collection, deg	2.39 – 23.39
F(000), e	2176
hkl range	± 31, $-11 \rightarrow 12$, ± 19
Refl. measured / unique / R_{int}	11979 / 1192 / 0.0696
$R1$ / $wR2$ [$I \geq 2\sigma(I)$]	0.0401 / 0.1087
$R(F)$ / $wR2$ (F^2) (all refl.)	0.0568 / 0.1155
GOF	0.956
$\Delta\rho_{fin}$ (max / min), e Å^{-3}	1.37 / −0.64

Table 2. Selected bond lengths (Å) and angles (deg) for complex **1**.

Sn(1)-O(2)	2.339(3)	Sn(1)-N(4)	2.451(4)
Sn(1)-O(1)	2.454(3)	Sn(1)-N(3)	2.345(4)
Sn(1)-C(9)	2.146(5)	C(3)-N(3)	1.312(6)
Sn(1)-C(15)	2.149(5)	S(1)-C(2)	1.730(5)
Sn(1)-S(1)	2.551(1)	N(3)–N(2)	1.362(5)
N(4)-Sn(1)-N(3)	68.00(1)	N(2)–C(2)	1.331(6)
N(3)-Sn(1)-S(1)	75.68(1)	O(2)-Sn(1)-S(1)	83.87(1)
C(9)-Sn(1)-C(15)	167.7(2)	O(1)-Sn(1)-N(4)	78.30(1)

Table 3. Hydrogen bond lengths (Å) and bond angles (deg) for complex **1**[a].

D–H···A	d(H···A)	d(D···A)	∠(D–H···A)
N(1)-H(1A)···O(2)$^{\#1}$	2.43	3.191(6)	150.2

[a] Symmetry operation: $^{\#1}$ $-x+1/2, y+1/2, -z+1/2$.

Results and Discussion

*Crystal and molecular structure of **1***

Table 1 summarizes crystal and refinement data for complex **1**. Selected bond lengths and angles are given in Table 2. Hydrogen bond lengths and angles are

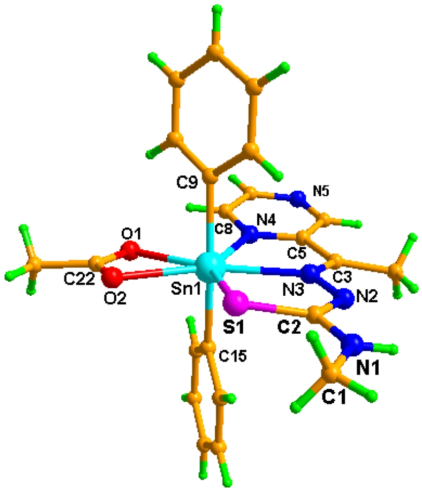


Fig. 1. Molecular structure of complex **1** with atomic numbering scheme adopted.

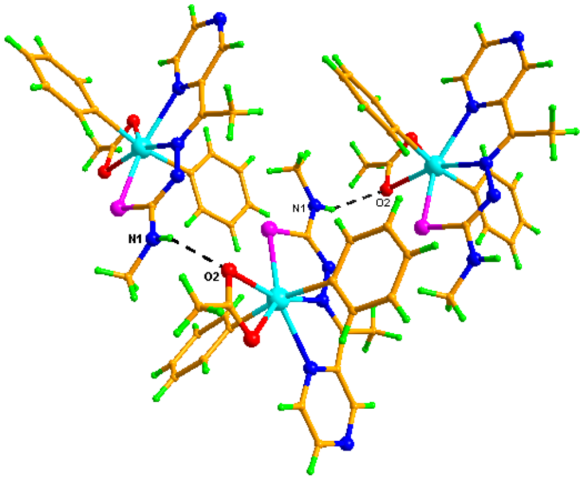


Fig. 2. Hydrogen bonds (dashed lines) in crystals of complex **1**.

listed in Table 3. The molecular and crystal structures along with the atom numbering scheme are depicted in Figs. 1 and 2, respectively.

As shown in Fig. 1 the tin atom in **1** is seven-coordinate and adopts a distorted pentagonal bipyramidal geometry with the pentagonal plane defined by the tridentate N_2S thiosemicarbazone and the bidentate acetato group, whereas the axial positions are occupied by the two phenyl groups. The distortion from pentagonal bipyramidal geometry is evident from the bond angles N(3)–Sn(1)–N(4) 68.00(1)°, N(3)–Sn(1)–S(1) 75.68(1)°, C(9)–Sn(1)–C(15) 167.7(2)°, O(2)-Sn(1)-S(1) 83.87(1)°, and O(1)-Sn(1)-N(4) 78.30(1)°. The pseudo-macrocyclic coordination mode of the ligand affords two five-membered chelate rings, which are nearly planar, the dihedral angle between the chelate rings being 4.5°. The C–S bond lengths of *ca.* 1.73 Å are within the normal range of C–S single bonds, indicating that the thiosemicarbazone moiety adopts the tautomeric thiol form and acts as a mononegative ligand [16a]. The C(3)–N(3) and N(3)–N(2) distances of 1.312(6) and 1.362(5) Å, respectively, are intermediate between formal single and double bonds, pointing to an extensive electron delocalization over the entire ligand skeleton. The shortening of the bond lengths of Sn–N (imine) relative to Sn–N (pyridine) may be attributed to the fact that the imine nitrogen is a stronger base compared with the pyridine nitrogen [19].

The molecules of complex **1** are held together in the crystal through intermolecular hydrogen bonds involving the terminal nitrogen atom N(1) of a thiosemicarbazone ligand and the oxygen atom O(2) of an acetato group (Fig. 2). The separation for N(1)···O(2) (symmetry code: $-x+1/2, y+1/2, -z+1/2$) is 3.191(6) Å with the N–H···O angle being 150.2°.

IR spectra

The infrared spectral bands most useful for determining the mode of coordination of the ligand are the ν(C=N), ν(N-N) and ν(C=S) vibrations. The IR spectrum of HL does not display ν(C–SH) in the region 2500 – 2600 cm^{-1} indicating that in the solid state these ligands remain in the thione form [20]. The ν(C=N) bands of HL at 1610 cm^{-1} are shifted to 1541 cm^{-1} in the spectrum of complex **1**, a clear sign of coordination *via* the imine nitrogen atom [21]. The band at 861 cm^{-1} in HL is assigned to ν(C=S), whereas in its complex the band is shifted to lower frequency (847 cm^{-1}), indicating the coordination of sulfur [22]. The increase in the frequency of the ν(N–N) band of the thiosemicarbazone in the spectrum of the complex is due to the increase in the bond strength, again confirming the coordination *via* the imine nitrogen. These observations were also evident from the above-described molecular structure determination.

In vitro cytotoxicity

In terms of the cytotoxic activity of thiosemicarbazones [23, 24], we have tested the ability of complex **1** as well as the starting compound Ph_2SnCl_2 to inhibit tumor cell growth against K562 leukaemia cells.

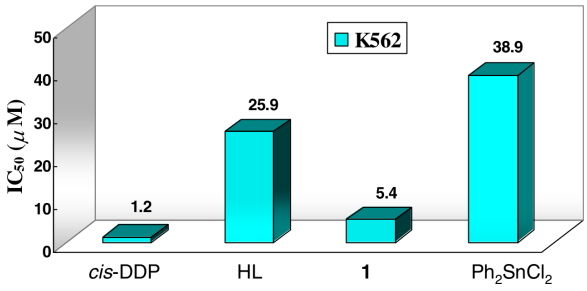


Fig. 3. The cytotoxicity of the tested compounds against the K562 leukaemia cell line [*cis*-DDP = *cis*-diamminedichloroplatinum(II)].

IC_{50} values (compound concentration that produces 50 % of cell death) in micromolar units were calculated (Fig. 3). The comparison of the cytotoxic activities indicates that **1** shows a much lower IC_{50} value (5.4 μM) than both HL (25.9 μM) [16c] and Ph_2SnCl_2 (38.9 μM) indicating that coupling of HL to Ph_2Sn(IV) leads to an enhancement of the antitumor activity of the free ligand. This confirms that complexation with a metal has a synergetic effect on the cytotoxicity [11, 16]. In particular, it should be emphasized that **1** shows activity comparable to that of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) (1.2 μM). Therefore, complex **1** merits further biological screening as well as studies of the mechanism of action.

Acknowledgement

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