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Synthesis, characterization, antibacterial, and cytotoxic activities of organotin(IV) complexes derived from *N*(4)-cyclohexylthiosemicarbazone: X-ray crystal structure of $[Ph_2SnCl(L)]$

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Reaction of organotin(IV) chloride(s) with 2-benzoylpyridine-*N*(4)-cyclohexylthiosemicarbazone, [HL] (**1**) yielded $[MeSnCl_2(L)]$ (**2**), $[BuSnCl_2(L)]$ (**3**), $[Me_2SnCl(L)]$ (**4**), and $[Ph_2SnCl(L)]$ (**5**). The ligand (**1**) and its organotin(IV) complexes have been characterized by CHN analyses, molar conductivity, UV-Vis, FT-IR, ^1H, ^{13}C, and ^{119}Sn NMR spectral studies. The molecular structure of **5** was also determined by X-ray diffraction. There are two independent molecules in the asymmetric unit and the central tin(IV) atom is six-coordinate in distorted octahedral geometry. The ligand (**1**) and complexes were screened for their *in vitro* antibacterial activities. The cytotoxic activities of **1**–**5** were tested against A2780 and A2780/Cp8 cancer cell lines. The compounds have better antibacterial activities than the free ligand; **2**–**5** are more potent cytotoxic agents than **1**, while the diphenyltin(IV) **5** is more active with IC_{50} values of 0.05 and 0.54 μmol L^{-1} against A2780 and A2780/Cp8 cell lines, respectively.

Keywords: Organotin(IV) complexes; Spectral analyses; Crystal structure; Antibacterial activity; Cytotoxicity

1. Introduction

Thiosemicarbazones are biologically important nitrogen–sulfur donors; metal complexes also show significant biological activity. Heterocyclic thiosemicarbazones chelate metals, coordinating in either neutral or deprotonated form. Suni *et al*. reported the synthesis and characterization of Co(II) and Co(III) complexes of di-2-pyridyl ketone

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N(4)-cyclohexyl and *N*(4)-phenyl thiosemicarbazones [1]. A huge amount of work on synthesis and characterization of transition metal complexes of thiosemicarbazone have been reported [2–6]. Tin complexes have multiple applications as antimicrobials and biocides [7, 8] with antiviral and antitumor activities and industrial and agricultural applications [9–12]. Antiproliferative and antitumor activities of organotin(IV) compounds have been reviewed [13] and applications of tin, including organotin, dithiocarbamates were reviewed by Tiekink [14]. Structural characteristics as determined by X-ray crystallographic methods were surveyed. Rebolledo *et al*. reported structural studies and cytotoxic activities of *N*(4)-phenyl-2-benzoylpyridine thiosemicarbazone tin(IV) complexes which suggested that the complexes were active as cytotoxic agents against MCF-7, TK-10, and UACC-62 human tumor cell lines [15]. The coordination chemistry of tin is extensive with various geometries and coordination numbers known for both inorganic and organometallic complexes [16, 17]. We reported synthesis and structural studies of organotin(IV) complexes with *N*(4)-substituted thiosemicarbazones which also displayed high antibacterial activities [18–20]. Herein, we report the synthesis and structural characterization of new organotin(IV) complexes (**2**–**5**) with 2-benzoylpyridine-*N*(4)-cyclohexylthiosemicarbazone, [HL] (**1**) and investigate their antibacterial and cytotoxic activities.

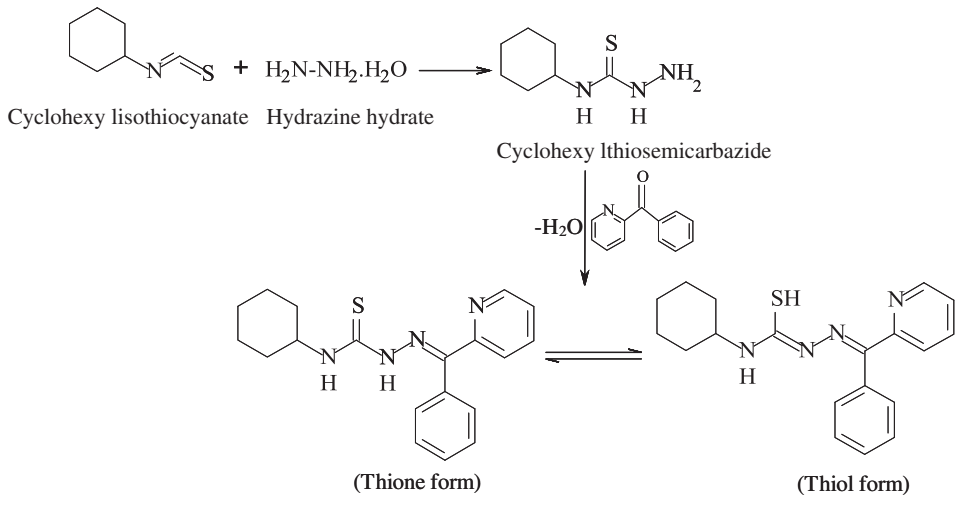
2. Experimental

2.1. *General procedure*

All reagents were purchased from Fluka, Aldrich, and JT Baker. Solvents were purified according to standard procedures [21]. UV-Vis spectra were recorded with DMSO on a Perkin Elmer Lambda 25 UV-Visible spectrophotometer. Infrared (IR) spectra were recorded on KBr discs using a Perkin Elmer Spectrum GX Fourier-Transform spectrometer from 4000 to 370 cm^{-1} at room temperature. ^{1}H, ^{13}C, and ^{119}Sn NMR spectra were recorded on a JEOL 500 MHz-NMR spectrometer; chemical shifts are given in ppm relative to $SiMe_4$ and Me_4Sn in DMSO. CHN analyses were obtained with a Flash EA 1112 series CHN elemental analyzer. Molar conductivity measurements were carried out with a Jenway 4510 conductivity meter using DMF. Single-crystal X-ray crystallographic analysis of **5** was carried out using an Oxford Diffraction Gemini CCD area-detector diffractometer with graphite monochromated Mo-Kα radiation ($\lambda = 0.71073$). The crystal structure was solved using direct methods (SIR92) and refined by full-matrix least-squares on F^2 using CRYSTALS Program Suite. Positional and anisotropic atomic displacement parameters were refined for all non-hydrogen atoms. Hydrogen atoms were positioned geometrically and refined with a riding model.

2.2. *Synthesis of N(4)-cyclohexylthiosemicarbazide*

Cyclohexylisothiocyanate (1.41 g, 10 mmol) in 4 mL of ether was added dropwise into 4 mL of an ether solution of hydrazine hydrate (2 g, 40 mmol). The mixture was stirred vigorously for 5 h. Then, 5 mL petroleum ether was added and stirred for another 1 h; a white precipitate formed. The white precipitate was filtered off, washed with a small amount of ether, and dried *in vacuo* over silica gel. Yield: 2.12 g, 62%; m.p.: 146–148°C;



Scheme 1. Synthesis of 2-benzoylpyridine-*N*(4)-cyclohexylthiosemicarbazone [HL, (**1**)].

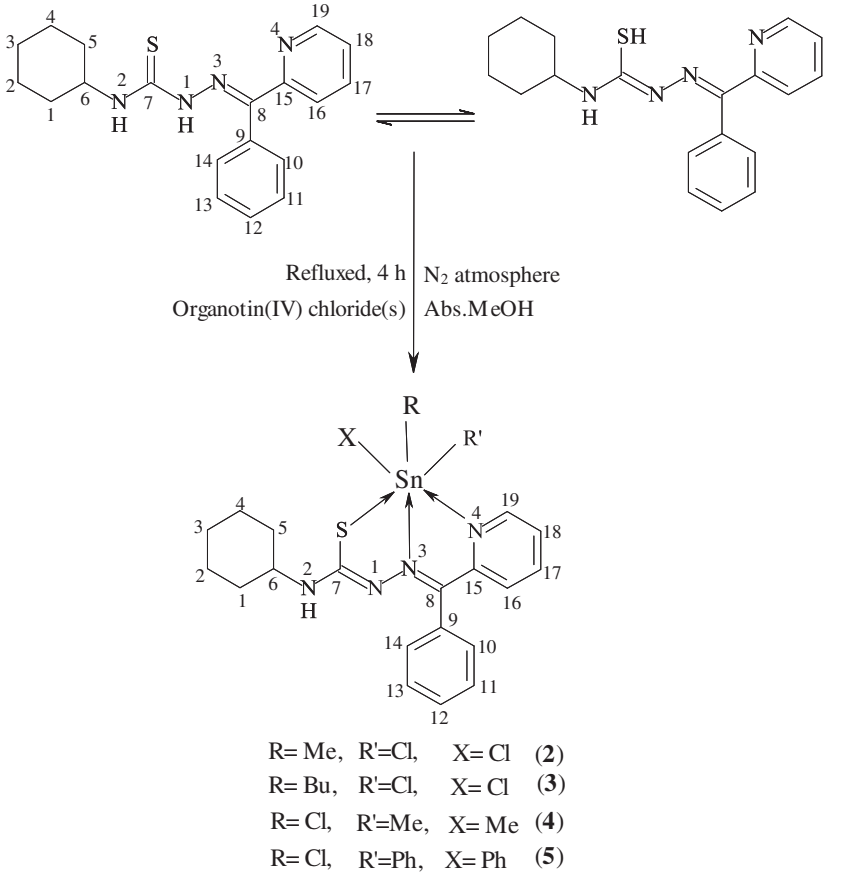
FT-IR (KBr disc, cm^{-1}) ν_{max}: 3334 (s, NH_2), 3297 (s, NH), 2929, 2853 (s, cyclohexyl), 1349, 849 (w, C=S).

2.3. *Synthesis of 2-benzoylpyridine-N(4)-cyclohexylthiosemicarbazone [HL, (1)]*

An absolute methanol solution of *N*(4)-cyclohexylthiosemicarbazide (0.51 g, 3 mmol) was refluxed with 2-benzoylpyridine (0.54 g, 3 mmol) in 10 mL absolute methanol continuously for 4 h after adding a few drops of acetic acid (scheme 1) and cooled to room temperature. White microcrystals were formed and filtered off. The microcrystals were washed several times with small amounts of absolute methanol and subsequently with cold hexane. The microcrystals were recrystallized from methanol and dried *in vacuo* over silica gel. Yield: 0.94 g, 89%; m.p.: 174–176°C: UV-Vis (DMSO) $\lambda_{max/nm}$: 280, 300, 347; FT-IR (KBr disc, cm^{-1}) ν_{max}: 3335 (s, NH), 2938, 2845 (s, cyclohexyl), 1583 (w, C=N), 984 (m, N–N), 1345, 863 (w, C=S), 608 (m, pyridine in plane). ^{1}H NMR (DMSO) δ: 11.54 (s, 1H, N1–H), 8.81 (d, 1H, pyridine ring C19–H), 7.76 (t, 1H, pyridine ring C17–H), 7.62 (d, 2H, 1H of N2–H, 1H of CyC6–H), 7.53 (d, 1H, pyridine ring C16–H), 7.48 (t, 1H, pyridine ring C18–H), 7.29–7.25 (m, 5H, phenyl ring), 2.11–1.71 (m, 10H, CyC–H). ^{13}C NMR (DMSO) δ: 206.97 (NH–C=S), 172.15 (C=N), 152.14–129.87 (pyridine ring carbon), 128.85–123.97 (phenyl ring carbon), 53.31–25.31 (cyclohexyl ring carbon). Anal. Calcd for $C_{19}H_{22}N_4S$ (%): C, 67.42; H, 6.55; N, 16.55. Found (%): C, 67.21; H, 6.31; N, 16.22.

2.4. *Synthesis of [MeSnCl$_2$(L)] (2)*

HL, (**1**) (0.34 g, 1.0 mmol) was dissolved in absolute methanol (10 mL) under a nitrogen atmosphere in a Schlenk round bottom flask. Then, an absolute methanol solution of methyltin(IV) trichloride (0.24 g, 1.0 mmol) was added dropwise, resulting in a yellow solution. The resulting reaction mixture was refluxed for 4 h (scheme 2) and cooled to



Scheme 2. The reaction scheme for syntheses of **2**–**5**.

room temperature. Yellow microcrystals, obtained from slow evaporation of the solution at room temperature, were filtered off, washed with a small amount of absolute methanol and dried *in vacuo* over silica gel. Yield: 0.48 g, 84%; m.p.: 202–204°C; molar conductance (DMF) $\Omega^{-1}\,cm^2\,mol^{-1}$: 11.3; UV-Vis (DMSO) $\lambda_{max/nm}$: 295, 334, 385, 416; FT-IR (KBr, cm^{-1}) ν_{max}: 3360 (s, NH), 2933, 2852 (s, cyclohexyl), 1596 (m, C=N–N=C), 1028 (w, N–N), 1308, 816 (m, C–S), 649 (w, pyridine in plane), 578 (w, Sn–C), 484 (w, Sn–N). ^{1}H NMR (DMSO, $^2J[^{119}Sn, ^1H]$) δ: 8.83 (d, 1H, pyridine ring C19–H), 8.01 (t, 1H, pyridine ring C17–H), 7.96 (d, 1H, pyridine ring C16–H), 7.66 (d, 2H, 1H of N2–H, 1H of CyC6–H), 7.51–7.25 (m, 6H, 1H of pyridine ring C18–H, 5H of phenyl ring), 2.15–1.73 (m, CyC–H), 1.08 (s, 3H, Sn–CH_3), [92.5 Hz]. ^{13}C NMR (DMSO, $[^1J(^{13}C–^{119}Sn])$ δ: 192.99 (N=C–S), 178.45 (C=N), 144.57–141.45 (pyridine ring carbon), 129.99–128.27 (phenyl ring carbon), 55.59–24.67 (cyclohexyl ring carbon), 17.31 (Sn–CH_3), [810.88 Hz]. ^{119}Sn NMR (DMSO) δ: −325.80. Anal. Calcd for $C_{20}H_{24}N_4SSnCl_2$ (%): C, 44.31; H, 4.46; N, 10.33. Found (%): C, 44.31; H, 4.38; N, 10.27.

Complexes **3**–**5** were synthesized using a similar procedure to **2** using appropriate organotin(IV) chloride(s) (scheme 2).

2.5. *Synthesis of [BuSnCl$_2$(L)] (3)*

Yield: 0.51 g, 81%; m.p.: 205–208°C; molar conductance (DMF) $\Omega^{-1}\,cm^2\,mol^{-1}$: 13.64; UV-Vis (DMSO) $\lambda_{max/nm}$: 293, 331, 385, 422; FT-IR (KBr, cm^{-1}) ν_{max}: 3353 (s, NH), 2929, 2852 (s, cyclohexyl), 1595 (m, C=N–N=C), 1073 (w, N–N), 1304, 815 (m, C–S), 648 (w, pyridine in plane), 580 (w, Sn–C), 485 (w, Sn–N). ^{1}H NMR (DMSO) δ: 8.82 (d, 1H, pyridine ring C19–H), 8.01 (t, 1H, pyridine ring C17–H), 7.94 (d, 1H, pyridine ring C16–H), 7.66 (d, 2H, 1H of N2–H, 1H of CyC6–H), 7.58–7.25 (m, 6H, 1H of pyridine ring C18–H, 5H of phenyl ring), 2.21–1.76 (m, 10H, CyC–H), 1.76–1.74 (t, 2H, Sn–CH_2–CH_2–CH_2–CH_3), 1.53–1.48 (m, 2H, Sn–CH_2–CH_2–CH_2–CH_3), 1.38–1.22 (m, 2H, Sn–CH_2–CH_2–CH_2–CH_3), 0.98–0.84 (t, 3H, Sn–CH_2–CH_2–CH_2–CH_3). ^{13}C NMR (DMSO, [^{1}J(^{13}C–^{119}Sn]) δ: 195.74 (N=C–S), 179.50 (C=N), 144.30–141.29 (pyridine ring carbon), 129.96–126.35 (phenyl ring carbon), 52.45–31.88 (cyclohexyl), 29.32, 27.06, 22.68, 14.07 (Sn–Bu) [806.05 Hz]. ^{119}Sn NMR (DMSO) δ: −310.40. Anal. Calcd for $C_{23}H_{30}N_4SSnCl_2$ (%): C, 47.38; H, 5.55; N, 9.60. Found (%): C, 47.31; H, 5.49; N, 9.53.

2.6. *Synthesis of [Me$_2$SnCl(L)] (4)*

Yield: 0.45 g, 81%; m.p.: 212–214°C; molar conductance (DMF) $\Omega^{-1}\,cm^2\,mol^{-1}$: 12.84; UV-Vis (DMSO) $\lambda_{max/nm}$: 299, 339, 381, 415; FT-IR (KBr, cm^{-1}) ν_{max}: 3335 (s, NH), 2939, 2852 (s, cyclohexyl), 1593 (m, C=N–N=C), 1074 (w, N–N), 1320, 833 (m, C–S), 652 (w, pyridine in plane), 531 (w, Sn–C), 479 (w, Sn–N). ^{1}H NMR (DMSO, ^{2}J[^{119}Sn, ^{1}H]) δ: 8.82 (d, 1H, pyridine ring C19–H), 8.55 (t, 1H, pyridine ring C17–H), 7.76 (d, 1H, pyridine ring C16–H), 7.63 (d, 2H, 1H of N2–H, 1H of CyC6–H), 7.47–7.25 (m, 6H, 1H of pyridine ring C18–H, 5H of phenyl ring), 2.15–1.63 (m, CyC–H), 1.17 (s, 6H, Sn–CH_3), [110 Hz]. ^{13}C NMR (DMSO, [^{1}J(^{13}C–^{119}Sn]) δ: 188.17 (N=C–S), 177.77 (C=N), 144.97–141.45 (pyridine ring carbon), 130.65–126.73 (phenyl ring carbon), 55.99–30.55 (cyclohexyl ring carbon), 17.77 (Sn–CH_3), [824.23 Hz]. ^{119}Sn NMR (DMSO) δ: −337.20. Anal. Calcd for $C_{21}H_{27}N_4SSnCl$ (%): C, 48.34; H, 5.21; N, 10.73. Found (%): C, 48.28; H, 5.14; N, 10.62.

2.7. *Synthesis of [Ph$_2$SnCl(L)] (5)*

Yield: 0.52 g, 76%; m.p.: 198–200°C; molar conductance (DMF) $\Omega^{-1}\,cm^2\,mol^{-1}$: 8.15; UV-Vis (DMSO) $\lambda_{max/nm}$: 295, 308, 367, 406; FT-IR (KBr, cm^{-1}) ν_{max}: 3379 (s, NH), 2929, 2851 (s, cyclohexyl), 1592 (m, C=N–N=C), 1020 (w, N–N), 1302, 811 (m, C–S), 636 (w, pyridine in plane), 588 (w, Sn–C), 457 (w, Sn–N). ^{1}H NMR (DMSO) δ: 8.82 (d, 1H, pyridine ring C19–H), 8.60 (t, 1H, pyridine ring C17–H), 8.55 (d, 1H, pyridine ring C16–H), 7.70 (d, 2H, 1H of N2–H, 1H of CyC6–H), 7.27–7.25 (m, 16H, 1H of pyridine ring C18–H, 15H of phenyl ring), 2.15–1.74 (m, 10H, CyC–H). ^{13}C NMR (DMSO, [^{1}J(^{13}C–^{119}Sn]) δ: 191.50 (N=C–S), 177.24 (C=N), 141.78–140.67 (pyridine ring carbon), 132.05–129.35 (phenyl ring carbon), 53.31–25.30 (cyclohexyl ring carbon), [984.45 Hz]. ^{119}Sn NMR (DMSO) δ: −321.30. Anal. Calcd for $C_{31}H_{31}N_4SSnCl$ (%): C, 57.65; H, 4.83; N, 8.67. Found (%): C, 57.34; H, 4.62; N, 8.48.

2.8. *Antibacterial test*

The antibacterial tests of **1**–**5** were carried out using the agar well diffusion method [22]. Doxycycline was used as standard drug. Bacteria from stock culture were lightly inoculated into the Mueller Hinton Broth (MHB) and allowed to grow overnight at 37°C in an ambient air incubator. The culture was diluted with new MHB in order to achieve optical density of 2.0×10^6 colony forming units ($CFU\,mL^{-1}$) or 0.168 at wavelength of 550 nm in the spectrophotometer. A sterile cotton swab was dipped into the broth culture and inoculated on the Mueller Hinton Agar (MHA). Sterile paper discs with 6 mm diameter were placed on the agar at equal distances. The recommended concentration of the test sample ($2\,mg\,mL^{-1}$ in DMSO) was introduced individually to each disc. The agar plates were incubated immediately at 37°C for 20 h. For each plate, DMSO mixture and reference antibacterial drug, doxycycline, serve as negative and positive controls, respectively. All tests were performed in triplicate with full agreement between the results. The activities were determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to positive control.

2.9. *Tumor cell lines*

A2780 and A2780/Cp8 cell lines, derived from an untreated patient with ovarian carcinoma, were maintained in the logarithmic phase at 37°C in a 5% CO_2 humidified atmosphere in air using RPMI 1640 medium supplemented with 10% fetal calf serum, $2\,\mu mol\,L^{-1}$ glutamine, $10\,mg\,mL^{-1}$ gentamycin, and $10\,\mu g\,mL^{-1}$ insulin.

2.10. *Cytotoxic effect*

The cytotoxic effects of **1**–**5** toward A2780 and A2780/Cp8 cells were evaluated by using the Cell Proliferation Kit I (MTT) of Boehringer-Mannheim, following the supplier's protocol. Briefly, the cells grown in the culture flasks were trypsinized and 100 μL of medium containing 5×10^4 cells was inoculated into 96-well microplates. After 24 h from seeding, the cells were incubated with various concentrations of organotin(IV) complexes freshly dissolved in DMSO ($1\,mg\,mL^{-1}$) and diluted in the culture medium (DMSO final concentration, 0.25%) for 3 days at 37°C. After an additional 4 h incubation with 10 μL per well of tetrazolium salt solution ($5\,mg\,mL^{-1}$), cells were dissolved in 100 μL of 10% SDS solubilization solution and the absorbance was measured at 570 nm. Control cells, treated with 0.25% DMSO in complete medium, did not show variation of either viability or cell proliferation with respect to cells seeded in drug-free medium. The drug concentration required to reduce cell number to 50% of controls following a 72 h continuous drug exposure (IC_{50}) was obtained from semilogarithmic dose-response plots. All tests were performed in triplicate with full agreement between the results.

3. Results and discussion

3.1. *Synthesis*

2-Benzoylpyridine-*N*(4)-cyclohexylthiosemicarbazone [HL, (**1**)] was prepared by condensation of 4-cyclohexylthiosemicarbazide and 2-benzoylpyridine (scheme 1); **1** is a mononegative tridentate NNS donor in this work. Reaction of organotin(IV) chloride(s) with **1** in 1 : 1 mole ratio leads to four organotin(IV) complexes, $[MeSnCl_2(L)]$ (**2**), $[BuSnCl_2(L)]$ (**3**), $[Me_2SnCl(L)]$ (**4**), and $[Ph_2SnCl(L)]$ (**5**) (scheme 2). The organotin(IV) complexes were obtained in good yield (76–84%), are stable under N_2, and soluble in $CHCl_3$, CH_2Cl_2, DMSO, and DMF. The physical and analytical data are given in section 2. The molar conductivity of **2**–**5** in DMF are 13.64–8.15 $\Omega^{-1}\,cm^2\,mol^{-1}$, indicating non-electrolytes [23].

3.2. *UV-Vis spectra*

UV-Vis spectra of **1** and **2**–**5** were carried out in DMSO (10^{-4} mol L^{-1}) at room temperature. Ligand **1** showed three absorptions at 280, 300, and 347 nm which correspond to HOMO/LUMO transitions of pyridyl ring, azomethine, and thiolato, respectively [24]. After complexation, the UV-Vis spectra of **2**–**5** showed absorption bands at 295–280, 339–300, 384–347, and 422–406 nm. The new absorptions at 422–406 nm were due to the n–π^* transition band of the ligand→metal (Sn) charge transfer [25]. The shift of the λ_{max} from the ligand to the complexes is a clear indication that coordination occurred between tin(IV) and **1**.

3.3. *IR spectra*

The IR spectrum of **1** shows a strong band at 3335 cm^{-1} attributed to NH linked to the cyclohexyl group. The other bands observed in the spectrum at 2938, 2845, 1583, 984, 1354, 863, and 608 cm^{-1} are due to ν(cyclohexyl), ν(C=N), ν(N–N), ν(C=S), and pyridine ring in plane, respectively. The absence of ν(S–H) at 2700 cm^{-1} suggests that **1** exists in the thione form in the solid state [26, 27]. After complexation, **2**–**5** show sharp bands at 1593–1596 cm^{-1} due to the newly formed ν(C=N–N=C) bond, indicating coordination of the azomethine nitrogen to tin(IV) [28]. The ν(N–N) of **1** at 984 cm^{-1} shifts to higher frequencies (1020–1074 cm^{-1}) in **2**–**5**, indicating coordination of azomethine nitrogen to Sn(IV). The stretching and bending frequency of ν(CS) at 1345 and 863 cm^{-1} in **1** shift to lower frequencies (1302–1320 cm^{-1} and 811–833 cm^{-1}) in spectra of **2**–**5**, indicating coordination of sulfur in the thiolate form upon deprotonation [29]. The in-plane deformation mode of the pyridine ring at 608 cm^{-1} in the spectrum of **1** shifts to 636–652 cm^{-1} in **2**–**5**, suggesting coordination of the pyridine ring nitrogen to tin(IV) [30]. In spectra of **2**–**5**, absorptions at 531–588 cm^{-1} were assigned to ν(Sn–C) and bands at 457–484 cm^{-1} were attributed to ν(Sn–N). IR spectra of **2**–**5** are in agreement with the X-ray data of **5**.

3.4. *^{1}H, ^{13}C, and ^{119}Sn NMR spectra*

^{1}H NMR spectra of **1** and **2**–**5** were interpreted based on the atom-labeling in scheme 2. In HL, a singlet at 11.54 ppm indicates N1–H. The pyridyl protons gave four

resonances, two doublets at 8.81 and 7.53 ppm correspond to PyC19–H and PyC16–H, respectively, while two triplets at 7.76 and 7.48 ppm belong to PyC17–H and PyC18–H, respectively. The proton resonance on N2–H and CyC6–H are a doublet at 7.62 ppm which is overlapping. The multiplets of aromatic-H in **1** were at 7.29–7.25 ppm. In the cyclohexyl, equatorial protons were at 2.11 ppm compared to their axial protons at 1.71 ppm. After complexation, ^{1}H NMR spectra of **2**–**5** undergo significant changes in chemical shifts as a result of coordination. The N1–H signal was absent in **2**–**5**, suggesting deprotonation and coordination of thiolate sulfur to tin(IV). After complexation, the PyC19–H signal is downfield at 8.82–8.83 ppm, indicating coordination of ligand to tin(IV) *via* pyridyl ring nitrogen (N4). The PyC18–H resonance probably overlaps with aromatic ring protons at 7.25–7.58 ppm in **2**–**5**, downfield compared to **1**. The resonances of PyC17–H and PyC16–H were between 8.01–8.60 and 7.76–8.55, downfield compared to **1**. This downfield shift supports pyridine nitrogen coordination with tin(IV). A doublet at 7.63–7.70 ppm in **2**–**5** corresponds to N2–H and CyC6–H overlapping. The [^{2}J(^{1}H, ^{119}Sn)] coupling satellites are clearly visible with 92.5 Hz for **2** and 110 Hz for **4**. The values for the [^{2}J(^{1}H, ^{119}Sn)] coupling constants were consistent with six-coordinate tin (IV) [31].

The ^{13}C NMR resonance signals of **1** were observed at 206.97, 172.15, 152.14–129.87 128.85–123.97, and 53.31–25.31 ppm due to the δ(NH–C=S), δ(C=N), δ(pyridine ring carbon), δ(phenyl ring carbon), and δ(cyclohexyl ring carbon), respectively. After complexation, signals of N=C–S shift upfield (195.74–188.17 ppm) in **2**–**5** compared to **1**, indicating coordination of deprotonated (N=C–S$^-$) to tin(IV). The chemical shift of C=N in **1** at 172.15 ppm shifts downfield to 179.50–177.24 ppm in **2**–**5**, indicating azomethine nitrogen is coordinated to tin(IV). Carbon signals of the pyridine ring in the free ligand were observed at 152.14–129.87 ppm, shifted upfield at 149.01–133.10 ppm in **2**–**5**, suggesting coordination of pyridine-N to tin(IV). Chemical shifts of aromatic ring and cyclohexyl ring carbons of **1** and **2**–**5** appear within the expected range. In ^{13}C NMR spectra, the phenyl and cyclohexyl carbon signals do not shift significantly on binding to tin(IV). The ^{1}J[^{119}Sn, ^{13}C] coupling constant is a key parameter in assessing coordination geometries of organotin(IV) compounds in solution. The ^{1}J[^{119}Sn, ^{13}C] coupling constants are 810.88, 806.05, 824.23, and 984.45 Hz for monomethyltin(IV) (**2**), monobutyltin(IV) (**3**), dimethyltin(IV) (**4**), and diphenyltin(IV) (**5**), respectively. These values are consistent with six-coordinate tin(IV) [31–33]. The C–Sn–C angle determination using the Lockhart–Manders equation [26] provided C–Sn–C angles of 146.06°, 149.3°, and 163.11° for monobutyltin(IV), dimethyltin(IV), and diphenyl(IV) complexes (**3**, **4**, and **5**), respectively. The observed C–Sn–C angles correlate with six-coordinate tin.

^{119}Sn NMR chemical shifts of **2**–**5** were recorded in $CDCl_3$ solution, giving significant information about coordination around tin. Complexes **2**–**5** show only one resonance at −310.40 to −337.20 ppm. The ^{119}Sn chemical shifts indicate six-coordinate organotin(IV) derivatives [34].

3.5. *X-ray crystallography diffraction*

The molecular structure of **5** with atom-numbering scheme is shown in figure 1. The main crystal parameters are reported in table 1 and selected bond lengths and angles are given in table 2. The compound crystallizes with two molecules per asymmetric unit into

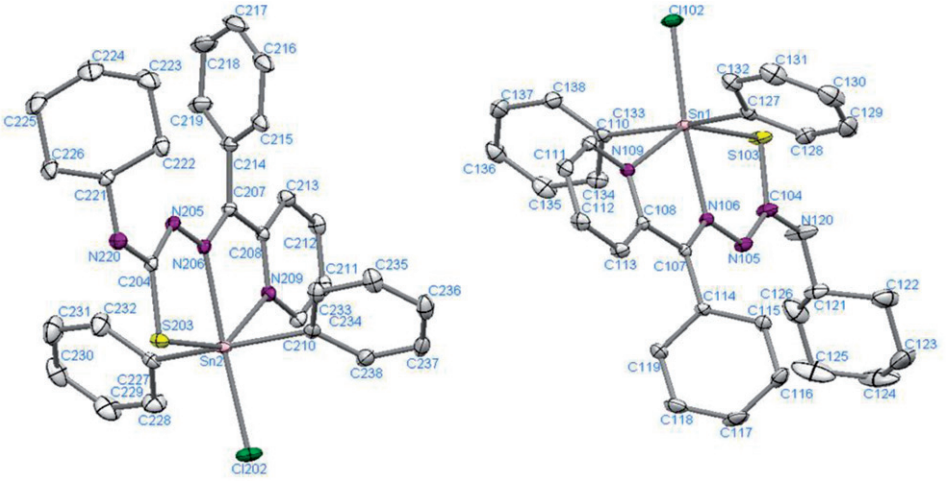


Figure 1. Molecular structure of **5**. Hydrogen atoms on cyclohexyl are omitted for clarity.

Table 1. Crystal data and structure refinement parameters for **5**.

Compound	$[Ph_2SnCl(L)]$ (**5**)
Empirical formula	$C_{31}H_{31}ClN_4SSn$
Formula weight	1291.64
Temperature (K)	150
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimensions (Å, °)	
a	10.90871(19)
b	13.6034(3)
c	21.4164(4)
α	105.0993(18)
β	103.8669(15)
γ	93.2745(16)
Volume (Å^3), Z	2945.62(11), 4
Calculated density (Mg m^{-3})	1.452
Absorption coefficient (μ) (mm^{-1})	1.052
Radiation type λ (Å)	Mo-Kα
F(000)	1312
Crystal size (mm^3)	0.060 × 0.012 × 0.180
Crystal color	Yellow
Scan range θ (°)	2.095–28.910
Max. and min. transmission	0.9874 and 0.9388
Data/restraints/parameters	11,761/0/685
Goodness-of-fit on F^2	0.9818
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0288$, $wR_2 = 0.0632$
R indices (all data)	$R_1 = 0.0385$, $wR_2 = 0.0679$

triclinic crystal system with a space group of $P\bar{1}$. From table 2, it is clear that the two molecules in the asymmetric unit are almost identical. So discussion can be limited to one of the molecules. Monodeprotonated ligand is coordinated to tin(IV) *via* pyridine-N, azomethine-N, and thiolato-S. The coordination geometry about Sn

Table 2. Selected bond lengths (Å) and angles (°) of **5**.

Sn1–Cl102	2.5341(6)	N105–N106	1.368(2)
Sn1–S103	2.5083(6)	N106–C107	1.302(3)
Sn1–N106	2.3209(17)	C107–C108	1.480(3)
Sn1–N109	2.3730(17)	C107–C114	1.483(3)
Sn1–C127	2.157(2)	N109–C108	1.347(3)
Sn1–C133	2.150(2)	C108–C113	1.392(3)
S103–C104	1.759(2)	N109–C110	1.338(3)
C104–N105	1.319(3)	N120–C121	1.454(3)
C104–N120	1.338(3)	C121–C122	1.520(4)
Cl102–Sn1–S103	99.00(2)	N109–Sn1–C127	82.15(7)
Cl102–Sn1–N106	174.00(4)	Cl102–Sn1–C133	90.12(6)
S103–Sn1–N106	75.97(4)	S103–Sn1–C133	98.39(6)
Cl102–Sn1–N109	115.45(4)	N106–Sn1–C133	93.85(7)
S103–Sn1–N109	145.53(4)	N109–Sn1–C133	83.30(7)
N106–Sn1–N109	69.57(6)	C127–Sn1–C133	163.11(9)
Cl102–Sn1–C127	88.41(6)	N109–C108–C113	120.44(19)
S103–Sn1–C127	98.46(6)	C108–N109–Cl10	119.64(19)
N106–Sn1–C127	89.09(7)	N109–C110–C111	122.6(2)

atom is best described as distorted octahedron. The equatorial positions are occupied by N109, N106, and S103 and remaining sites are occupied by chloride (Cl102) and two carbon atoms (C127 and C133) from phenyls. The two carbon atoms are axial, while chloride (Cl102) is *trans* to N106. Chloride (Cl102) and N106 show slightly longer bond lengths [d(Sn1–Cl102) = 2.5341(6) Å] and [d(Sn1–N106) = 2.53209(17) Å] than the two axial carbons of phenyl groups [d(Sn1–C127) = 2.157(2) Å] and [d(Sn1–C133) = 2.150(2) Å]. The Sn1–N106 and Sn1–N109 bond distances are 2.3209(17) Å and 2.3730(17) Å, significantly different, probably resulting from the geometry of **1**, indicating strong tin–nitrogen interaction [35]. The Sn1–S103 bond distance is 2.5083(6) Å, close to the sum of covalent radii of Sn–S (2.42 Å) [36], but much smaller than the sum of the van der Waals radii (4.0 Å) [37]. The N106–C107 and N105–C104 bond distances are 1.302(3) Å and 1.319(3) Å, respectively, in conformity with a formal C=N double bond (1.28 Å). The values are similar, indicating thiol tautomeric form. Therefore, C104–S103 bond changes from double bond to predominately single bond whereas C104–N105 bond acquires some double bond character. The chloride (Cl102) and nitrogen (N106) in axial positions Cl102–Sn1–N106 angle is 174.0(4)°, showing distortion from linear. The coordination geometry about the Sn(IV) is distorted octahedral with two phenyl groups in axial positions with a C127–Sn1–C133 angle of 163.11(9)°, deviating from linearity, due to the steric hindrance of the bulky phenyls. The S103–Sn1–N109 bond angle (145.53(4)°) is much smaller than expected. The sum of the equatorial angles S103–Sn1–N106 (75.97(4)°), N106–Sn1–N109 (69.57(6)°) and S103–Sn1–N109 (145.53(4)°) is 291.07° showing large distortion from ideal 360°. The sum of the angles S103–Sn1–Cl102 (99.00(2)°), S103–Sn1–N106 (75.97(4)°), N106–Sn1–N109 (69.57(6)°), and N109–Sn1–Cl102 (115.45(4)°) is 360°. Thus the atoms Sn1, Cl102, N106, and N109 are coplanar. The angles C110–N109–C108 (119.64(19)°), N109–C110–C111 (122.6(2)°), C110–C111–C112 (118.4(2)°), C111–C112–C113 (119.7(2)°), C112–C113–C108 (119.2(2)°), and C113–C108–N109 (120.44(19)°) are not 120°; thus, all the atoms in pyridyl ring are not the same.

Table 3. Antibacterial activities[a] of **1** and **2–5** (inhibition zone in mm).

Compound	Zone of inhibition (mm)			
	S. aureus	*E. aerogenes*	*E. coli*	*S. typhi*
HL (**1**)	–	12.3	11.2	9.5
n-$BuSnCl_3$	12.8	12.5	12.9	12.6
2	17.5	19.4	12.6	14.1
3	18.7	20.3	17.8	15.5
4	18.2	2.6	19.9	20.4
5	22.5	24.2	21.1	23.6
R	31.7	32.2	28.5	26.9

R = Standard drug: Doxycycline; dash indicates inactivity.
[a]Concentration used: 2 mg mL^{-1} in DMSO.

3.6. *Antibacterial activity*

Ligand **1** and complexes **2–5** were tested against *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Salmonella typhi* to assess their antibacterial activities. The results of the antibacterial studies are given in table 3 along with the values for [*n*-$BuSnCl_3$]. The concentration (2 mg mL^{-1} in DMSO) was used for each respective test sample. The results were compared with standard drug (doxycycline). *n*-$BuSnCl_3$ shows similar antibacterial activity against all the bacterial species at inhibition zones of 12.9–12.5 mm. HL shows moderate activity toward the bacteria, while **2–5** show high activity against various bacteria but lower activity than the reference drug. The antibacterial activity shown by these compounds against all bacteria indicates that complexes of HL (**1**) with R/R_2Sn(IV) results in complexes with biological properties; complexes **2–5** exhibit more inhibitory effects than the parent ligand or starting organotin(IV) chloride (*n*-$BuSnCl_3$). The activity of **2–5** can be attributed to the bulky cyclohexyl substituent that is a good electron donating group, increasing the lipophilic character of the complexes by strengthening metal ligand bonds [38–41]. Comparable activities of **2** and **3** may be due to the presence of NH. Of the four complexes studied, **5** showed the highest inhibition to growth of organisms, i.e., 21.1, 22.5, 24.2, and 23.6 mm inhibition zones against *E. coli*, *S. aureus*, *E. aerogenes*, and *S. typhi*, respectively; the presence of phenyl groups facilitate binding to biological molecules by π-interaction. This antibacterial result is corroborated to those previously reported for other tin compounds [42–44]. Antibacterial activity of compounds is due to either bactericide effects (killing the bacteria) or bacteriostatic effects (inhibiting multiplication of bacteria by blocking their active sites) [45].

3.7. *Cytotoxic activity*

Ligand **1** and complexes **2–5** were tested for cytotoxic activity *in vitro* against A2780 and A2780/Cp8 ovarian cancer cell lines. The effect of **2–5** was evaluated in comparison to that of *cisplatin* and **1** in a pair of *cisplatin*-sensitive and *cisplatin*-resistant ovarian cancer cell lines (A2780 and A2780/Cp8, respectively, table 4). Complexes **2–5** show a cytotoxic effect toward A2780 and A2780/Cp8. Even **1** showed marked cytotoxicity toward A2780 and A2780/Cp8 with IC_{50} values being 0.38 and 0.90 μmol L^{-1}, respectively. The IC_{50} values for **2** against A2780 and A2780/Cp8 are

Table 4. Cytotoxic effects (IC_{50}, $\mu mol\,L^{-1}$)[a] of **1** and **2–5** toward A2780 and A2780/Cp8 ovarian cancer cell lines.[b]

No.	Compound	A2780	A2780/cp8
1	HL	0.38 ± 0.20	0.90 ± 0.35
2	$[MeSnCl_2(L]$	0.30 ± 0.02	0.94 ± 0.25
3	$[BuSnCl_2(L)]$	0.32 ± 0.06	0.58 ± 0.05
4	$[Me_2SnCl(L)]$	0.05 ± 0.03	0.50 ± 0.19
5	$[Ph_2SnCl(L)]$	0.05 ± 0.02	0.54 ± 0.17
R	Cisplatin	2.28 ± 0.97	6.67 ± 1.7

[a]Mean ± SD, $n > 3$ experiments.
[b]72 h exposure, MTT assay.

0.30 and 0.94 $\mu mol\,L^{-1}$, respectively, 8 and 7 times more active than *cisplatin* against A2780 and A2780/Cp8, respectively. Complex **3** is 7 and 11 times more active than *cisplatin* against A2780 and A2780/Cp8 cell lines, respectively; complex **4** is 10 and 19 times more active than *cisplatin* against A2780 and A2780/Cp8, respectively, and complex **5** is 45 and 13 times more active than *cisplatin* against A2780 and A2780/Cp8, respectively. Selectivity was observed for **2–5**. The higher cytotoxicity of **2–5** can be explained on the basis of high lipophilic character. Cytotoxic activities of **2–5** are presumably due to the presence of pyridyl to form hydrogen bonds with DNA bases [41]. The higher cytotoxicity of **5** may be due to the presence of phenyls, which facilitate binding to biological molecules by π–π interactions. Complexes **2–5** show cytotoxic potency in a very low micromolar range, better than the antitumor drug *cisplatin*. The activities of **4** and **5** against two ovarian cancer cell lines (table 4) show them to be very efficient anticancer agents. These results stimulate further investigations of organotin(IV) complexes with substituted thiosemicarbazone ligands, in particular on the spectrum of their antitumor as well as on the mechanistic properties underlying their ability of overcoming *cisplatin* resistance.

4. Conclusion

Organotin(IV) complexes **2–5** of 2-benzoylpyridine-*N*(4)-cyclohexylthiosemicarbazone (HL) have been synthesized and characterized. HL exists in the thione form in the solid state, but takes on a thiol form in solution. Complexes **2–5** are six-coordinate, coordinated through thiolato-S, azomethine-N, and pyridine ring-N. These compounds show significant antibacterial activities and were active against A2780 and A2780/Cp8 cancer cell lines. The compounds are biologically active, with **5** more active than **2–4**. Coordination of organotin(IV) salts with HL could be an interesting strategy for further stages of screening *in vitro* activities.

Supplementary material

CCDC reference number 795060 contains the supplementary crystallographic data for $[Ph_2SnCl(L)]$ (**5**). These data can be obtained free of charge from the Cambridge Crystallographic Data Center *via* www.ccdc.cam.ac.uk/data_request/cif or from the

Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (+44) 1223 336 033; or E-mail: deposit@ccdc.cam.ac.uk.

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