DALTON

# Synthesis, crystal structure and biological activity of bis(acetone thiosemicarbazone-*S*)dichlorodiphenyltin(IV)

**Siang-Guan Teoh,*[a] Show-Hing Ang,[a] Soon-Beng Teo,[a] Hoong-Kun Fun,[b] Khing-Ling Khew[c] and Chi-Wi Ong[d]**

[a] *School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*
[b] *X-Ray Crystallographic Laboratory, School of Physics, Universiti Sains Malaysia, 11800 Penang, Malaysia*
[c] *School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*
[d] *Department of Chemistry, National Sun Yat-Sen University, Kaohsiung, Taiwan 804, Republic of China*

The reaction between diphenyltin(IV) dichloride and thiosemicarbazide using acetone–ethanol as solvent resulted in the formation of bis(acetone thiosemicarbazone-*S*)dichlorodiphenyltin(IV), $SnPh_2Cl_2(atsc)_2$. The crystal structure determination of the compound revealed it to be a monomeric six-co-ordinated organotin(IV) complex. Each of the two atsc functions as a monodentate ligand, co-ordinating to the tin atom through the sulfur atom and conferring a distorted-octahedral geometry upon the tin. The Sn–S bond length is 2.712(1) Å. The antifungal activity of the complex, atsc and $SnPh_2Cl_2$ against four plant pathogens has been evaluated. The complex displays marked fungitoxicity against these fungi and is more fungitoxic than free atsc and $SnPh_2Cl_2$. It has also shown significant cytotoxicity against human colon adenocarcinoma, breast adenocarcinoma, hepatocellular carcinoma and acute lymphoblastic leukaemia.

Interest in the co-ordination chemistry of thiosemicarbazones has increased since their biological activity was shown to be related to their metal complexing ability.[1] A large number of reports describing the transition-metal complexes of these compounds have appeared.[2–5] However, little is known about the corresponding complexing behaviour of non-transition elements such as tin.[2,6–8]

In this paper we report the synthesis, crystal structure and antifungal and anticancer activities of bis(acetone thiosemicarbazone-*S*)dichlorodiphenyltin(IV), $SnPh_2Cl_2(atsc)_2$, obtained from the reaction between diphenyltin(IV) dichloride and acetone thiosemicarbazone (atsc) derived *in situ* from thiosemicarbazide and acetone.

## Experimental

### General and instrumental

Thiosemicarbazide obtained from Fluka Chemie AG was used after purification from ethanol. Diphenyltin dichloride was used as obtained from Aldrich and the solvents used were reagent grade.

Carbon, hydrogen and nitrogen analyses were carried out on a Control Equipment Corporation 240XA elemental analyser at the School of Chemical Sciences, Universiti Sains Malaysia, Penang, Malaysia. The tin analysis was performed using an Instrumental Laboratory aa/ee 357 atomic spectrophotometer. The IR spectrum was recorded using a Mattson 1000 FTIR spectrophotometer in the frequency range 4000–200 $cm^{-1}$ with the sample in a KBr disc, the $^1H$ NMR spectrum on a Bruker 300 MHz AC-P spectrometer in $(CD_3)_2SO$ solution.

### Synthesis of bis(acetone thiosemicarbazone-*S*)dichlorodiphenyltin(IV)

A solution of $SnPh_2Cl_2$ (1 mmol) in ethanol (20 $cm^3$) was added to a hot solution of thiosemicarbazide (2 mmol) in a mixture of acetone (10 $cm^3$) and ethanol (10 $cm^3$). The mixture was heated moderately (≈50 °C) and stirred for 45 min. The resulting solution was allowed to stand and slow evaporation at room temperature gave light yellow crystals which were stable in air. Yield 78%, m.p. 164–165 °C (Found: C, 39.4; H, 4.5; N, 12.9; Sn, 18.8. Calc. for $C_{20}H_{28}Cl_2N_6S_2Sn$: C, 39.65; H, 4.65; N, 13.85; Sn, 19.6%). IR(KBr): 1595 (C=N) and 770 $cm^{-1}$ (C–S), $^1H$ NMR [$(CD_3)_2SO$]: δ 9.89 (s, 1 H, NH), 7.92–7.26 (m, 5 H, $C_6H_5$), 7.97 (s, 1 H, $NH_2$), 7.50 (s, 1 H, $NH_2$) and 1.90 (d, 6 H, $2CH_3$).

### Crystallography

A needle-shaped single crystal of dimensions 0.2 × 0.4 × 0.5 mm was mounted on a thin glass fibre on a Siemens $P_4$ diffractometer equipped with graphite-monochromated Mo-Kα radiation, λ = 0.710 73 Å, $T$ = 298 K. The θ–2θ scan method was employed to measure a total of 3819 reflections in the $3.0 \leqslant 2\theta \leqslant 55.0°$ shell. Corrections were applied for Lorentz-polarization effects but not for absorption. There were 2988 independent reflections of which 2530 satisfied the $F > 4.0\sigma(F)$ criterion of observability and were used in the subsequent analysis. The structure was solved using direct methods and refined by a full-matrix least-squares procedure based on $F$ using SHELXTL (PC version).[9] All non-hydrogen atoms were refined using anisotropic thermal parameters and hydrogen atoms were placed in calculated positions (C–H 0.96, N–H 0.90 Å) and refined isotropically. A weighting scheme of the form $w = [\sigma^2(F) + 0.0024F^2]^{-1}$ was used and the refinement continued to final $R = 0.0258$ and $R' = 0.0280$. The final difference map had peaks between −0.87 and 0.54 e $Å^{-3}$. The crystal data and refinement parameters are given in Table 1, bond distances and angles in Table 2.

Atomic coordinates, thermal parameters, and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1997, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 186/348.

**Table 1** Crystal data and refinement details for $SnPh_2Cl_2(atsc)_2$

| | |
|---|---|
| Formula | $C_{20}H_{28}Cl_2N_6S_2Sn$ |
| $M$ | 606.2 |
| Crystal system | Monoclinic |
| Space group | $P2_1/c$ |
| $a$/Å | 9.3770(10) |
| $b$/Å | 13.7470(10) |
| $c$/Å | 10.8690(10) |
| β/° | 111.640(10) |
| $U$/Å$^3$ | 1302.3(2) |
| $Z$ | 2 |
| $D_c$/Mg m$^{-3}$ | 1.546 |
| μ/mm$^{-1}$ | 1.366 |
| $F$(000) | 612 |
| $h,k,l$ Ranges | −1 to 12, −1 to 17, −14 to 13 |
| Reflections collected | 3819 |
| Independent reflections | 2988 ($R_{int}$ = 0.0211) |
| Observed reflections | 2530 [$F > 4.0\sigma(F)$] |
| No. parameters refined | 198 |
| $R$ | 0.0258 |
| $R'$ | 0.0280 |

**Table 2** Bond lengths (Å) and angles (°) with estimated standard deviations in parentheses for non-hydrogen atoms

| | | | |
|---|---|---|---|
| Sn–Cl(1) | 2.589(1) | Sn–S(1) | 2.712(1) |
| Sn–C(1) | 2.142(3) | Sn–Cl(1A) | 2.589(1) |
| Sn–S(1A) | 2.712(1) | Sn–C(1A) | 2.142(3) |
| S(1)–C(7) | 1.724(2) | C(1)–C(2) | 1.386(4) |
| C(1)–C(6) | 1.396(3) | C(2)–C(3) | 1.391(6) |
| C(3)–C(4) | 1.372(6) | C(4)–C(5) | 1.377(7) |
| C(5)–C(6) | 1.398(6) | C(7)–N(1) | 1.313(3) |
| C(7)–N(2) | 1.327(2) | C(8)–C(9) | 1.491(4) |
| C(8)–C(10) | 1.503(5) | C(8)–N(3) | 1.278(3) |
| N(2)–N(3) | 1.396(3) | | |
| | | | |
| Cl(1)–Sn–S(1) | 88.8(1) | Cl(1)–Sn–C(1) | 90.0(1) |
| S(1)–Sn–C(1) | 92.4(1) | Cl(1)–Sn–Cl(1A) | 180.0(1) |
| S(1)–Sn–Cl(1A) | 91.2(1) | C(1)–Sn–Cl(1A) | 90.0(1) |
| Cl(1)–Sn–S(1A) | 91.2(1) | S(1)–Sn–S(1A) | 180.0(1) |
| C(1)–Sn–S(1A) | 87.6(1) | Cl(1A)–Sn–S(1A) | 88.8(1) |
| Cl(1)–Sn–C(1A) | 90.0(1) | S(1)–Sn–C(1A) | 87.6(1) |
| C(1)–Sn–C(1A) | 180.0(1) | Cl(1A)–Sn–C(1A) | 90.0(1) |
| S(1A)–Sn–C(1A) | 92.4(1) | Sn–S(1)–C(7) | 105.8(1) |
| Sn–C(1)–C(2) | 120.2(2) | Sn–C(1)–C(6) | 120.3(2) |
| C(2)–C(1)–C(6) | 119.4(3) | C(1)–C(2)–C(3) | 120.6(3) |
| C(2)–C(3)–C(4) | 120.1(4) | C(3)–C(4)–C(5) | 119.8(6) |
| C(4)–C(5)–C(6) | 121.0(4) | C(1)–C(6)–C(5) | 119.0(3) |
| S(1)–C(7)–N(1) | 120.7(2) | S(1)–C(7)–N(2) | 120.5(2) |
| N(1)–C(7)–N(2) | 118.8(2) | C(9)–C(8)–C(10) | 117.6(2) |
| C(9)–C(8)–N(3) | 126.8(3) | C(10)–C(8)–N(3) | 115.6(3) |
| C(7)–N(2)–N(3) | 118.1(2) | C(8)–N(3)–N(2) | 116.4(2) |

### Antifungal test

Fungitoxicity is usually measured in terms of the response of a treated culture relative to that of a control. In this assay, a 100 ppm stock of compound in PDA medium was prepared by dissolving the compound (0.01 g) in $Me_2SO$ (1 cm$^3$), adjusting the volume to 100 cm$^3$ with PDA (potatoes dextrose agar) medium. From the stock different concentrations (50, 10, 5 and 1 ppm) were prepared. The treated medium and control were then poured into five sterilized petri dishes respectively and allowed to set for 24 h before being inoculated with the fungus. As soon as the fungal colony covered the whole plate of the control medium the colony diameters of all the control and treated plates were measured. The areas of fungal growth were calculated by using the average diameters of the fungal colony. The fungal growth area was plotted against the compound concentration and the $ED_{50}$ value was obtained as 50% of the largest fungal growth area at a certain compound concentration. The antifungal activities of the complex, the free thiosemicarbazone and the tin starting compound are summarized in Table 3.

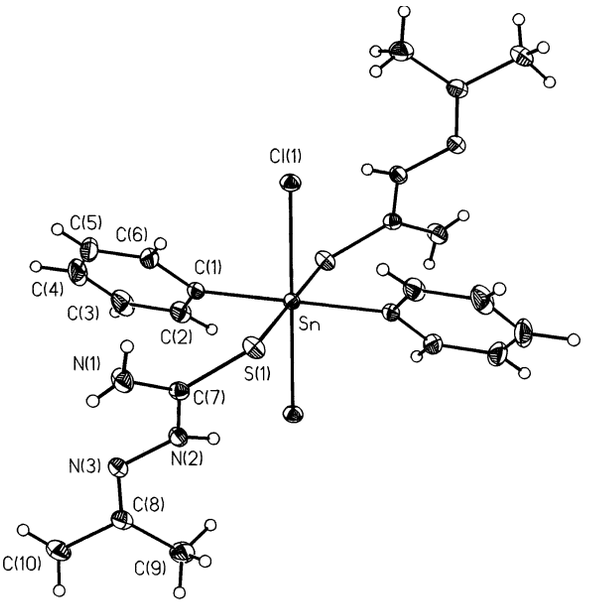



**Fig. 1** Molecular structure with atom labelling for $SnPh_2Cl_2(atsc)_2$

### Cytotoxicity assay: MTT assay

In principle, this assay is dependent on the cellular reduction of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] by the mitochondrial dehydrogenase of viable cells to give a blue formazan product. The viable cell number per well is directly proportional to the production of formazan, which following solubilization can be measured spectrophotometrically.

Single-cell suspensions were obtained by mechanical disaggregation of the floating cell line for human acute lymphoblastic leukaemia (MOLT-4) and by trypsinization of the monolayer cultures for human colon adenocarcinoma (COLO-205), breast adenocarcinoma (SKBR-3) and hepatocellular carcinoma (HA22T/VGH) and counted by trypan blue exclusion. The cells were then planted on to 96 well plates (Nunc 67008) in a volume of 180 μl using a multichannel pipette (Gilson) and incubated for 24 h.

The compound was dissolved in 10% $Me_2SO$ and 90% DPBS (Dulbecco's phosphate-buffered saline) solution. The 20 μl solution was dispensed within appropriate wells (each treatment group and control, $N = 3$) to give final concentrations ranging from 100 to 0.01 μg/cm$^{-3}$ by a 10-fold dilutions. Peripheral wells for each plate (lacking cells) were utilized for drug blank and medium/tetrazolium reagent blank 'background determinations'. The cells were then incubated for 72 h.

A 20 μl volume of MTT (5 mg cm$^{-3}$) was added to each well and incubated for 4 h. Culture plates containing suspension lines or any detached cells were centrifuged at low speed (1000 revolutions min$^{-1}$) for 5 min. The 170 μl culture medium supernatant was removed from each well and replaced with 200 μl $Me_2SO$ per well using a multichannel pipette. Following thorough formazan solubilization (vibration on a plate shaker), the absorbance of each well was measured using an ELISA automatic plater (Molecular Devices *Emax*) at 545–690 nm interfaced with IBM computer Softmax software. Cell growth inhibition was calculated according to [1 − (*A* upon compound treatment/*A* of control)] × 100%. The $IC_{50}$ was obtained as the 50% growth inhibition at a certain compound concentration from a plot of the compound concentration *vs.* percentage growth inhibition. The values of $IC_{50}$ of the complex and adriamycin (as a reference) are shown in Table 4.

## Results and Discussion

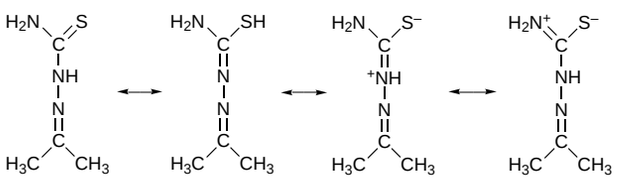
As shown in Fig. 1 the structure of bis(acetone thio-

**Table 3** Antifungal activity

| | $ED_{50}$/μg $cm^{-3}$ | | | |
|---|---|---|---|---|
| Compound | *Curvularia* sp. | *Drechsiera* sp. | *Rhizoctonia* sp. | *Alternaria Bassicicola* sp. |
| $SnPh_2Cl_2(atsc)_2$ | 2.15 | 0.50 | 2.05 | 2.80 |
| atsc | >100 | >100 | >100 | 145 |
| $SnPh_2Cl_2$ | 10 | 9 | >100 | 24 |

**Table 4** Cytotoxic activity

| | $IC_{50}$/μg $cm^{-3}$ | | | |
|---|---|---|---|---|
| Compound | COLO-205 | HA22T/VGH | SKBR-3 | MOLT-4 |
| $SnPh_2Cl_2(atsc)_2$ | 4.62 | 3.76 | 1.44 | 0.50 |
| Adriamycin | 0.470 | 0.300 | 0.053 | <0.001 |




semicarbazone-*S*)dichlorodiphenyltin(IV) shows a distorted octahedron about the tin atom which is co-ordinated to two phenyl, two chloride and two acetone thiosemicarbazone (atsc) groups. Each of the atsc ligands co-ordinates to the tin atom in the *trans* configuration,[10,11] and therefore it behaves as a monodentate ligand, bonding only through the sulfur atom. The deviation from octahedral symmetry is indicated by the bond angles subtended at the tin atom by adjacent donor atoms, ranging from 88.8(1)° for Cl(1)–Sn–S(1) and Cl(1A)–Sn–S(1A) to 92.4(1)° for S(1)–Sn–C(1) and S(1A)–Sn–C(1A).

A comparison of bond distances with those of atsc,[12] $[W(CO)_5(atsc)]$[1] and $[Ni(atsc - H)_2]$[13] can be made. The thiosemicarbazone acts as a monodentate ligand in $[W(CO)_5(atsc)]$, bonded through the sulfur atom, whereas in $[Ni(atsc - H)_2]$, both of the atsc ligands are bonded through S and hydrazine N atoms. The N(2)–N(3) [1.396(3) Å] bond distance in the present compound is close to N(1)–N(2) [1.398(6) Å] in free atsc and N(2)–N(3) [1.396(8) Å] in $[W(CO)_5(atsc)]$, but shorter than the average N–N bond length [1.425(7) Å] in $[Ni(atsc - H)_2]$. This indicates that co-ordination from N(3) to the tin atom does not occur in the present complex as in $[W(CO)_5(atsc)]$.

The C(7)–S(1) [1.724(2) Å] bond length is shorter than the average C–S bond distance in $[Ni(atsc - H)_2]$. This is because in the latter deprotonation of atsc has taken place before co-ordination through the sulfur anion and then formation of a C–S single bond. In the present complex, the atsc ligands co-ordinate to the tin atom without deprotonation but in the zwitterion resonance forms shown.[14] Owing to resonance the C–S bond possesses partial double-bond character[15] and hence it is shorter. However, it is longer than the C–S distance in free atsc [1.690(5) Å], indicating that co-ordination causes an increase in the C–S single-bond character.[16]

The Sn–S [2.712(1) Å] bond length is longer than that observed for triphenyltin 1-amino-4-(2-hydroxyphenyl)-2,3-diazapenta-1,3-diene-1-thiolate[17] [2.440(2) Å]. This can be explained from the bond types. For the present compound the Sn–S bond exists as a weak dative bond while for the latter tin complex it is as a covalent bond.

It is of interest that the $^1H$ NMR spectrum of the complex exhibits two resonances for the $NH_2$ protons (at δ 7.50 and 7.97), indicating hindered rotation about the C(S)–$NH_2$ bond due to its partial double-bond character.[18,19] The presence of the imine proton at δ 9.89 shows that deprotonation does not take place during complexation.

Results of the *in vitro* antifungal bioassay indicate that the metal complex is more fungitoxic than is free atsc and the parent organotin compound. It proved to have a very strong activity against the four fungi tested especially *Drechsiera* sp. where the minimum inhibitory concentration is only 0.50 μg $cm^{-3}$.

On co-ordination the tin atom is firmly attached to the ligand and its positive charge is shared with the donor groups (S atoms). As a result the complex reduces the polarity of the metal ion and, in turn, increases its hydrophobic character and thus promotes its permeation through the lipoid layers of the fungus membranes.[20]

Thiosemicarbazones have been demonstrated to show biological activity against viruses, protozoas, pathogens and certain kinds of tumours.[21] The fungicidal activity of thiosemicarbazones is due basically to their ability to chelate the necessary metals which the fungus requires in its metabolism.[22] However, acetone thiosemicarbazone did not display any fungitoxicity against the fungi tested. One possible explanation is that there is no extensive delocalization involving the alkyl group $CH_3$ and the thiosemicarbazide side chain.

From the anticancer screening data presented in Table 4 it is obvious that the complex showed remarkable cytotoxicity as an $IC_{50}$ value less than 4 μg $cm^{-3}$ normally is considered to represent activity. Interestingly the compound is most active against leukaemia where the $IC_{50}$ value is only 0.5 μg $cm^{-3}$.

## Acknowledgements

S.-G. T., S.-B. T. and H.-K. F. would like to thank the Malaysian Government and Universiti Sains Malaysia for R & D Grants Nos. 190/9609/3406 and 190/9609/2801.

## References

1 J. Valdes-Martinez, A. Sierra-Romero, C. Alvarez-Toledano, R. A. Toscano and H. Garcia-Tapia, *J. Organomet. Chem.*, 1983, **352**, 321.
2 Kiran, R. V. Singh and J. P. Tandon, *Synth. React. Inorg. Metal-Org. Chem.*, 1986, **16**, 1341.
3 M. Nath, N. Sharma and C. L. Sharma, *Synth. React. Inorg. Metal-Org. Chem.*, 1989, **19**, 339.
4 V. K. Arora, K. B. Pandeya and R. P. Singh, *J. Indian Chem. Soc.*, 1979, **56**, 656.
5 P. L. Maurya, B. V. Agarwala and A. K. Dey, *J. Indian Chem. Soc.*, 1980, **57**, 275.
6 A. K. Saxena, J. K. Koacher and J. P. Tandon, *J. Inorg. Nucl. Chem.*, 1981, **43**, 3091.
7 A. Saxena and J. P. Tandon, *Polyhedron*, 1984, **3**, 681.
8 A. Varshney and J. P. Tandon, *Polyhedron*, 1986, **5**, 739.
9 G. M. Sheldrick, SHELXTL PC™, Release 4.1, Siemens Crystallographic Research Systems, Madison, WI, 1990.
10 M. J. M. Campbell, *Coord. Chem. Rev.*, 1975, **15**, 279.
11 S. Padhye and G. B. Kauffman, *Coord. Chem. Rev.*, 1985, **63**, 127.
12 G. J. Palenik, *Acta Crystallogr., Sect. B*, 1974, **30**, 2390.
13 J. Valdes-Martinez, R. A. Toscano, M. Soriano-Garcia, M. Rubio, J. Gomez-Lara, M. A. Vazquez-M and S. Carranza, *Polyhedron*, 1989, **8**, 727.
14 R. Restivo and G. J. Palenik, *Acta Crystallogr., Sect. B*, 1970, **26**, 1397.

15 M. K. Kokila, Puttaraja, M. V. Kulkarni and S. Thampi, *Acta Crystallogr., Sect. C*, 1995, **51**, 330.
16 M. Mathew and G. J. Palenik, *J. Am. Chem. Soc.*, 1969, **91**, 6310.
17 S. W. Ng, V. G. Kumar Das, B. W. Skelton and A. H. White, *J. Organomet. Chem.*, 1989, **377**, 211.
18 C. Bellito, D. Gattegno, A. M. Giuliani and M. Bossa, *J. Chem. Soc., Dalton Trans.*, 1976, 758.
19 P. Umpathy, A. P. Budhkar and C. S. Dorai, *J. Indian Chem. Soc.*, 1986, **63**, 714.
20 T. T. Bamgboye and O. A. Bamgboye, *Inorg. Chim. Acta*, 1987, **133**, 247.
21 K. N. Thimmaiah, G. T. Chandrappa, Rangaswamy and Jayarama, *Polyhedron*, 1984, **3**, 1237.
22 A. Albert and R. J. Goldere, *Nature* (*London*), 1948, **161**, 95.

*Received* 13*th August* 1996; *Paper* 6/05679B