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New mononuclear diorganotin(IV) dithiocarboxylates: synthesis, characterization and study of their cytotoxic activities

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Since organotin complexes have been reported to show fewer side effects relative to other heavy metal anticancer compounds, in the present study we report for the first time four novel organotin(IV) derivatives with the general formula R_2SnL_2, where R = methyl (1), *n*-butyl (2), phenyl (3), benzyl (4) and L = morpholine-1-carbodithioate (MCDT). The newly synthesized ligand was monodentate or bidentate, coordinating through a sulfur atom. The complexes were synthesized by directly mixing, refluxing and stirring the ligand, with diorganotin(IV) dichlorides in a suitable solvent. The complexes were found to be pure and their solid and solution phase structural configuration was investigated by FT-IR, multinuclear NMR (^{1}H, ^{13}C, ^{119}Sn) and mass spectrometry. Complex 2 was also studied for its thermal decomposition by thermogravimetry and differential thermal analysis. The results obtained on the basis of these techniques are in full concurrence with the proposed 1:2 (Sn:L) stoichiometry. The cytotoxic activity of the MCDT and diorganotin(IV) complexes (1–4) was tested against tumor cell lines – human cervix carcinoma HeLa and human myelogenous leukemia K562 – and normal immunocompetent cells: peripheral blood mononuclear cells PBMC. Results of bioassay demonstrated that organotin derivatives were in general more active than the anticancer drug cisplatin. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: tin compound; dithiocarboxylate; spectroscopy; antitumor activity

Introduction

The fight against cancer is one of the most important targets concerning medicinal chemistry, in which bioorganometallic chemistry has become an interesting research field.[1] Initial efforts in the evaluation of platinum-based anticancer drugs have been shifted to non-platinum metal-based agents with positive, no or limited side effects in advanced clinical trials.[2] Recent studies have shown very promising antitumor activity of organotin compounds against a wide panel of tumors or tumor cell lines, both *in vivo* and *in vitro*.[3] A careful choice of the ligand coordinated to an organotin(IV) fragment can modulate the cytotoxic activity of the organotin (IV) complex and minimize its drawbacks. Dithiocarboxylates anions are among the most well-known coordinating agents capable of binding nearly all metal ions.[4] The resonance due to these anions is the significant contribution from dithiocarboxylates to stabilize the overall electronic structure (Scheme 1).

From a biochemical point of view these ligands play a notable role in medicine also. For instance, the diethyldithiocarbamate anion, $Et_2CNS_2^-$ has been extensively used as an antidote for copper poisoning, i.e. Wilson's disease,[5] and ameliorating nephrotoxicity associated with platinum-based chemotherapy.[6] An important class of compounds are the mono-, di-, and triorganotin dithiocarbamates, which have been studied in solution and by single-crystal X-ray diffraction analysis.[7,8] The dithiocarboxylate complexes of organotin have generated interest because of their structural diversity, antitumor activity and enormous number of biological applications.[9,10] Organotin complexes are involved in cancer treatment via different mechanistic pathways at the molecular level. It is generally accepted that DNA is the most important intracellular target of anticancer drugs. The binding ability of organotin compounds towards DNA depends on the coordination number and nature of organic groups bonded to the central tin atom. The phosphate group of nucleotides usually acts as an anchoring site and nitrogen of DNA base binding is quite common, this often resulting in the stabilization of the tin center as an octahedral stable species.[11–17] Therefore, synthesis of novel organotin dithiocarboxylates with different structural features is a research area of increasing interest in inorganic, pharmaceutical and medicinal chemistry as a possible approach to the development of new drugs and in other areas and applications.[4] Encouraged by these findings and our interest in the field of organotin complexes, we synthesized some novel organotin compounds obtained by the interaction of a number of organotin(IV) halides with sulfur donor ligand. The complexes have been characterized by FT-IR, NMR (^{1}H, ^{13}C, ^{119}Sn), and mass spectrometry. Thus, as a continuation of our work, we decided to evaluate the cytotoxic activity of organotin(IV) dithiocarboxylate derivatives in order to observe the influence of substituents attached to the central tin atom on final antitumor activity.

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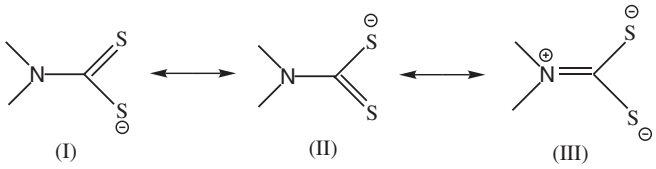
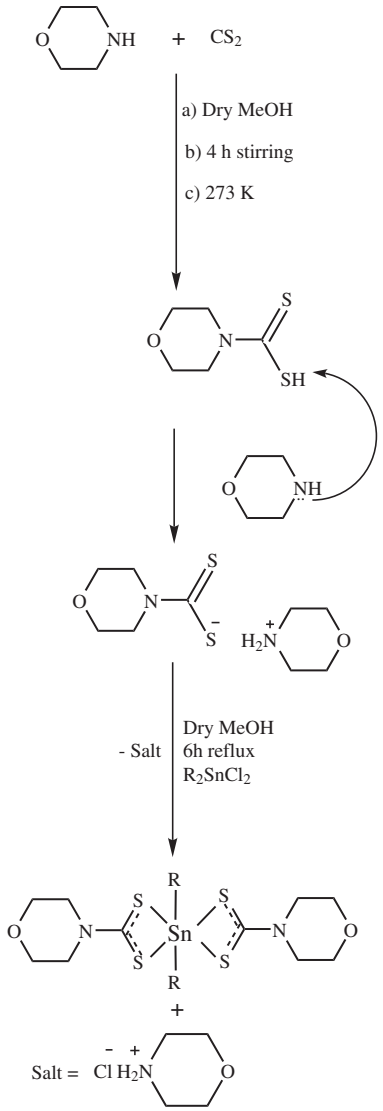


Scheme 1. Resonant forms of the -NCSS- moiety.

Results and Discussion

synthesis of Ligand Salt and Complexes 1–4

Nucleophilic attack of morpholine on carbon disulfide gave morpholine-1-carbodithioic acid as an intermediate which undergoes acid–base reaction with unreacted morpholine to give the ligand salt. The reaction of R_2SnCl_2 (R = Me, *n*-Bu, Ph, Bz) with the ligand salt in a ratio of 1:2 gave R_2SnL_2 and the same by-product as shown in Scheme 2. The numbering scheme of ligand and organic groups attached to Sn atom is given in Scheme 3.



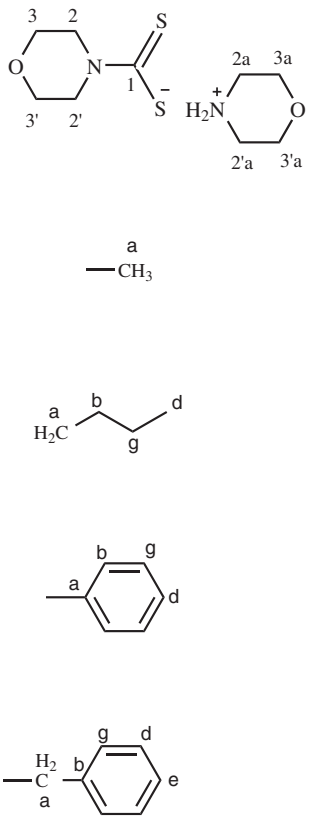
R= CH_3 (1), C_4H_9 (2), C_6H_5 (3), CH_2-C_6H_5 (4)

Scheme 2. Synthetic mechanism of ligand-salt and complexes.

Infrared Spectroscopy

The characteristic IR frequencies (cm^{-1}) of ligand and its diorganotin(IV) derivatives **1–4** are listed in Table 1. In the spectra of the investigated compounds, the vibration modes C-N and CS_2 are of particular interest in differentiating between monodentate and bidentate coordination of the 1,1-dithiolate moiety. The presence of a single band in the region of 900–1000 cm^{-1} due to ν(C-S) is an indication of bidentate character, while the splitting of this bond into a doublet with separation value > 20 cm^{-1} suggests that ligand bonding is monodentate.[18] In our case the bond at 1019 cm^{-1} assignable to a ν(C-S) in the ligand shifts to lower frequencies of ~1000, 995, 996, and 997 cm^{-1} in complexes (**1–4**), respectively. The stretching vibration peaks of the C-N in the studied compounds were located at 1450–1482 cm^{-1}.

These values lie between the range of C-N single bonds (1250–1360 cm^{-1}) and C=N double bond (1640–1690 cm^{-1}), which is an indication of the partial double bond character in the C-N bond.[19]



Scheme 3. Numbering scheme of ligand-salt and organic groups.

Table 1. Diagnostic IR bands of the ligand and its complexes.

Compound	ν(Sn-S)	ν(Sn-C)	ν(C. . .S)	ν(C. . .N)
MCDT	—	—	1019	1450
Compound **1**	413	540	1000	1468
Compound **2**	418	540	996	1465
Compound **3**	449	543	995	1482
Compound **4**	417	544	997	1474

^{1}H NMR Spectra

The ^{1}H NMR spectra of the investigated compounds were recorded in DMSO, taking tetramethylsilane (TMS) as an internal standard. The assignment of the proton resonances was made by their peak multiplicity, intensity pattern, and comparison of integration values of the protons with the expected molecular composition of the compounds. For the ligand, the morpholine protons demonstrated four triplets at 4.35, 3.77, 3.60 and 3.07 ppm in the aliphatic region, as expected for the structure. The ^{1}H NMR spectra of compound **1** exhibit a sharp singlet at 1.57 ppm corresponding to the protons of methyl groups attached to the Sn atom.[20] In addition, satellite signals of these protons, due to coupling with ^{117}Sn and ^{119}Sn isotopes, were observed. The coordination around Sn atom was deduced from [$^{2}J(^{119}Sn, H)$] coupling constant for compound **1**; the value observed was 82, thus confirming six coordinated Sn atoms. However, in the case of compound **2**, two sets of signals were observed at 2.1–1.45 and 0.97 ppm for the butyl group, respectively. As the *n*-butyl group is attached to an electropositive Sn atom via carbon nuclei, a shielding effect is experienced through the carbon chain.[21]

The signals for the protons of the phenyl groups attached to the Sn were distinguishable as two sets. The *ortho* protons were observed downfield (7.93 ppm) and these for *meta* and *para* protons upfield (7.42 ppm) in compound **3**. In complex **4** the protons of the benzyl part resonated in two regions: a singlet (3.2 ppm) due to CH_2 protons and two sets (7.15, 7.02 ppm) due to the phenyl moiety. In compounds **1–4** integration values demonstrate the attachment of two ligands to an Sn atom.

^{13}C NMR Spectra

^{13}C NMR data have been recorded for the ligand and its complexes and these spectra also support the authenticity of the proposed structures. The ^{13}C NMR chemical shifts due to methyl, *n*-butyl, phenyl, and benzyl groups attached to an Sn atom were observed at positions comparable to the other similar compounds.[22,23]

The signals due to the CS_2 carbon atom in the ligand appear at 213.6 ppm. However, in the spectra of the corresponding tin complexes, these appear at 199.2, 201, 196.7 and 199 ppm, respectively. In complexes **1–4** upfield shifts were observed, indicating the coordination of ligand to the Sn center via the CSS moiety.[24] In organotin(IV) complexes **2**, **3** and **4** the coupling constant at one bond distance, between the ^{13}C and ^{117}Sn and ^{119}Sn nuclei, was not observed owing to the low intensity of these signals, even when recording the spectra at very long relaxation delays. In order to provide further structural evidence, which establishes the structure of the complex in solution, we recorded ^{119}Sn NMR spectra.

^{119}Sn NMR Spectra

^{119}Sn NMR spectroscopy has been found to be quite useful for elucidation of the nature of the coordination of organotin(IV) to dithiocarbamates.[25] Even though for each complex with the same coordination number, some wide range of δ (^{119}Sn) values ore observed depending on the different organic and dithiocarboxylate groups attached to the Sn atom, there is an approximate linear relationship between the ^{119}Sn values and the coordination number of the complexes. According to Holecek and coworkers, for the range of +200 to −60, −90 to −190, −210 to −400, −440 to −540 ppm, the coordinate number of the tin is four, five, six and seven, respectively.[26–30] The appearance of a single peak in the ^{119}Sn NMR spectra of all the studied complexes signified the formation of single species.[31] The ^{119}Sn NMR chemical shift values for diorganotin complexes (**1–3**) were found in the range −225 to −326 ppm, indicating the tin atoms are six-coordinated in the studied complexes.

However, the ^{119}Sn signal for **4** lies at lower frequencies (−484 ppm) than that for six-coordinate complexes of dibenzyltin derivatives. This observation suggests that, in DMSO solution, the solvent may increase the coordinate state of complex **4**.

Mass Spectrometry

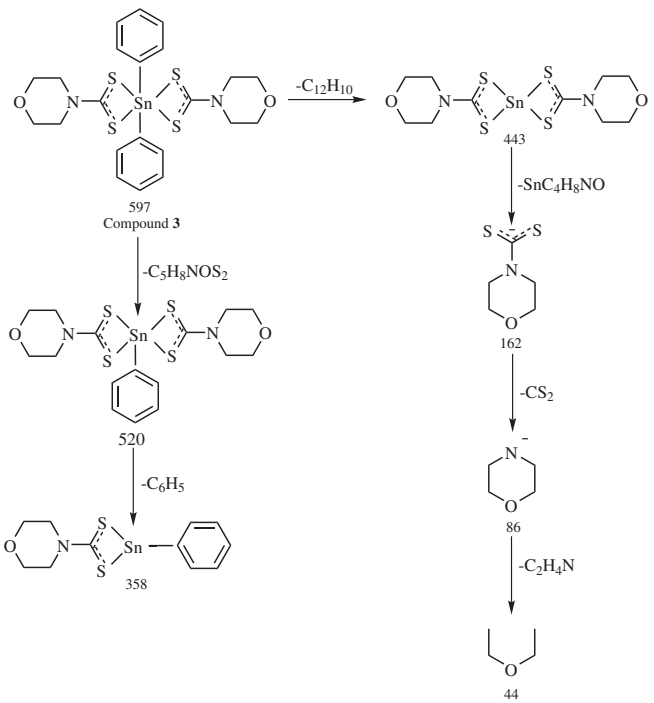
The conventional mass spectral data for compounds **1–4** are reported and different fragmentation patterns have been proposed. The typical feature of the mass spectra of organotin compounds is the cleavage of the most labile bond in the molecule to yield two complementary ions. The possible fragments of compound **3** are given in Scheme 4.

Thermal Studies

Thermogravimetric analysis (TGA) of compound **2** has been carried out to study the pyrolysis pattern in the temperature range 25–580 °C. The TG curve indicates decomposition to be a three-step process and the final residue weight corresponding to the metal oxide. The first step corresponds to the loss of water; the second decomposition step of the compounds exhibits 58% weight loss corresponding to the decomposition of morpholine-1-carbodithioate; and the third thermolitic stage accounts for the degradation of butyl groups.

Cytotoxic Studies

The *in vitro* cytotoxicities of morpholinium morpholine-1-carbodithioate (MCDT) as well as corresponding diorganotin(IV) complexes (**1–4**) against tumor cell lines – human cervix carcinoma HeLa and human myelogenous leukemia K562 – and on normal



Scheme 4. Fragmentation pattern of compound 3.

immunocompetent cells – peripheral blood mononuclear cells (PBMC) – were determined by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) microculture colorimetric assay. This study has been carried out in order to understand the possible relationship between the different tin(IV) moieties (bearing methyl, butyl, phenyl and benzyl groups), dithiocarboxylate ligand and the cytotoxic activity. The results of cytotoxic activity *in vitro* are expressed as IC_{50}, the concentration of compound ($\mu g\,ml^{-1}$) that inhibits proliferation rate of the tumor cells by 50% as compared to control untreated cells (Table 2).

Table 2. IC_{50} ($\mu g\,ml^{-1}$) for the 72 h of action of the studied compounds and cisplatin on HeLa, K562 and PBMC stimulated with PHA determined by MTT test.

Compound	HeLa	K562	PBMC + PHA
MCDT	0.38	0.031	0.99
Compound **1**	0.09	0.16	0.01
Compound **2**	0.13	0.17	0.11
Compound **3**	0.15	0.17	0.02
Compound **4**	0.17	0.02	0.02
Cisplatin[a]	1.32	1.71	7.8

[a]Standard drug, *cis*-$[Pt(NH_3)_2(Cl)_2]$.

Analyzed the free ligand and organotin complexes showed a dose-dependent antiproliferative effect towards all cell lines and on stimulated PBMC (Fig. 1).

Estimates based on the IC_{50} values show that the ligand is less cytotoxic compared to diorganotin (IV) complexes and more cytotoxic than cisplatin against the investigated tumor cell lines and on stimulated PBMC. The investigated organotin(IV) anticancer agents present lower IC_{50} values than those of cisplatin, which indicates their high activity against the tumor cell lines. The most outstanding results were obtained from the activity of compound **4** (IC_{50} 0.02 $\mu g\,ml^{-1}$), which is 85 times better than that of cisplatin against K562 cell line. It should be noted that presence of benzyl moiety in the structure of complex **4** had a great influence on the growth suppression activity of this complex on K562 cells. The IC_{50} values of complexes **1**, **2** and **3** against K562 cell line were in a similar range, and were 10 times better than that of the antitumor drug cisplatin. Complex **1** exhibited high activity against the HeLa cell line (IC_{50} 0.09 $\mu g\,ml^{-1}$) and moderate activity (IC_{50} 0.16 $\mu g\,ml^{-1}$) against the K562 cell line. This result emphasizes the importance of the methyl moiety that is present in complex **1**. In order to determine the selectivity in the *in vitro* cytotoxicity of complexes **1–4**) some additional experiments were conducted on stimulated PBMCs. The substitution of phenyl and benzyl groups on the tin atom of compounds **3** and **4** have similar effects on the antiproliferative activity on stimulated PBMC (IC_{50} 0.02 $\mu g\,ml^{-1}$).

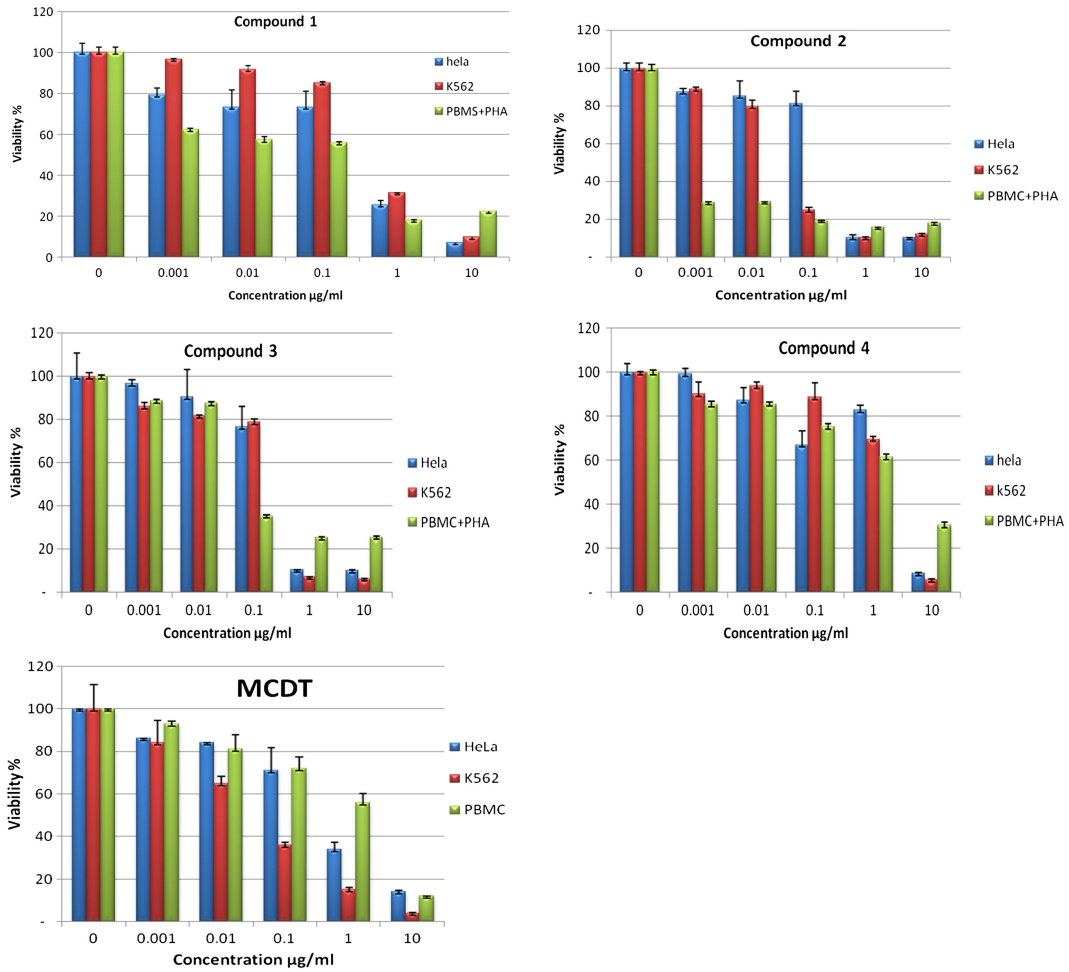


Figure 1. Cytotoxicity graphs from typical MTT assays showing the effect of MCDT and diorganotin(IV) complexes on the viability of K562, HeLa and PBMC + PHA (PBMC stimulated with PHA) cells.

Complex **1** is the most active against stimulated PBMC (IC_{50} 0.01 μg ml^{-1}) compared to activity on other cell lines used in this study. The cytotoxic activity shown by these compounds against all these cancer cell lines indicates that coupling of ligand with $R_2Sn(IV)$ metal center results in metallic complexes with important biological properties and remarkable cytotoxic activity. Compounds **1** and **4** are considered agents with potential antitumor activity and can therefore be candidates for further stages of screening *in vitro* and/or *in vivo*.

Experimental

Materials and Methods

All chemicals and reagents purchased were of reagent grade and used without further purification unless otherwise noted. Dibenzyltin(IV) dichloride was prepared by a standard method reported in the literature.[32] The solvents were purified and dried according to standard procedure. Melting points were determined in open capillaries and were uncorrected. FT-IR spectra were recorded on a Bomem MB-100 FT-IR spectrometer, using KBr pellets (400–4000 cm^{-1}). The ^{1}H, ^{13}C and ^{119}Sn NMR were obtained using a Bruker AVANCE 500 spectrometer. The chemical shifts are reported in ppm relative to the internal references, TMS for ^{1}H, ^{13}C and tetramethyltin for ^{119}Sn shifts. The splitting of proton resonances in the reported ^{1}H NMR spectra are defined as s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet. TGA was performed with a universal V3.8 B TA SDT Q500. Mass spectroscopy was performed on a Hewlett-Packard 5973 instrument at 70 eV.

Synthesis of morpholinium morpholine-1-carbodithioate (MCDT)

Dropwise addition of CS_2 (in excess, 1.7 ml, 23 mmol) in methanol (30 ml) to morpholine (1 ml, 11.5 mmol) in methanol (30 ml) followed by stirring for 4 h at 0 °C gave the white product. This was filtered off and washed with diethyl ether. M.p. 195 °C; FT-IR (KBr): ν = 1019 (C-S), 1450 (C-N) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 4.35 (t, $H_{3,3'}$, 4 H, $^3J_{H\text{-}H}$ = 10 Hz), 3.77 (t, $H_{3a,3'a}$, 4 H, $^3J_{H\text{-}H}$ = 10 Hz), 3.60 (t, $H_{2,2'}$, 4 H, $^3J_{H\text{-}H}$ = 10 Hz), 3.07 (t, $H_{2a,2'a}$, 4 H, $^3J_{H\text{-}H}$ = 10 Hz) ppm; ^{13}C NMR (500 MHz, DMSO): δ = 213.6 (C-1), 66.94 (C-3,3′), 64.23 (C-3a,3′a), 50.68 (C-2,2′), 43.71 (C-2a,2′a) ppm.

*Synthesis of dimethyltin(IV)-bis(morpholine-1-carbodithioate) (**1**)*

A solution of Me_2SnCl_2 (0.1 g, 0.45 mmol) in methanol (15 ml) was added dropwise to a solution of ligand (0.22 g, 0.9 mmol) in methanol (30 ml) at 60 °C. The reaction mixture was refluxed for 6 h with constant stirring and the solvent was evaporated; then the colorless crystals were separated out 5 days later. M.p. 162 °C; FT-IR (KBr): ν = 1000 (C-S), 1468 (C-N), 413 (Sn-S), 540 (Sn-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 1.57 (s, H_α ,6 H, $^2J_{Sn\text{-}H}$ = 82 Hz), 4.13 (t, $H_{3,3'}$, 8 H, $^3J_{H\text{-}H}$ = 10 Hz), 3.79 (t, $H_{2,2'}$, 8 H, $^3J_{H\text{-}H}$ = 10 Hz) ppm; ^{13}C NMR (500 MHz, DMSO): δ = 199.2 (C-1), 66 (C-3,3′), 51.25 (C-2,2′), 12.77 (C-α, $^1J_{C\text{-}Sn}$ = 700 Hz) ppm; ^{119}Sn NMR (500 MHz, DMSO): δ = −322 ppm; MS: *m/z* (70 eV) = 209.7 $(C_2S_2H_3Sn)^+$, 162 $(C_5H_8NOS_2)^+$, 133 $(CH_3Sn)^+$, 130 $(C_5H_8NOS)^+$, 86 $(C_4H_8NO)^+$, 72 $(C_4H_8O)^+$, 58 $(NCS)^+$.

*Synthesis of dibutyltin(IV)-bis(morpholine-1-carbodithioate) (**2**)*

The preparation of compound **2** was carried out in an identical manner to compound **1**. Bu_2SnCl_2 (0.1 g, 0.33 mmol) and ligand salt (0.16 g, 0.66 mmol). M.p. 140 °C; FT-IR(KBr): ν = 996 (C-S), 1465 (C-N), 418 (Sn-S), 543 (Sn-C); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.16 (t, $H_{3,3'}$, 8 H, $^3J_{H\text{-}H}$ = 10 Hz), 3.79 (t, $H_{2,2'}$, 8 H, $^3J_{H\text{-}H}$ = 10 Hz), 2.11–1.45 (m, $H_{\delta\ ,\beta,\gamma}$, 12 H), 0.97 (t, H_α, 6 H, $^3J_{H\text{-}H}$ = 15 Hz) ppm; ^{13}C NMR (500 MHz, $CDCl_3$): δ = 201 (C-1), 66.1 (C-3,3′), 51.2 (C-2,2′), 26.3 (C_δ), 28.4 (C_β), 34.19 (C_γ), 13.7 (C_α) ppm; ^{119}Sn NMR (500 MHz, $CDCl_3$): δ = −326 ppm; MS: *m/z* (70 eV) = 395 $(C_{13}H_{26}NOS_2Sn)^+$, 280 $(C_5H_8NOS_2Sn)^+$, 162 $(C_5H_8NOS_2)^+$, 130 $(C_5H_8NOS)^+$, 86 $(C_4H_8NO)^+$, 77 $(C_6H_5)^+$.

*Synthesis of diphenyltin(IV)-bis(morpholine-1-carbodithioate) (**3**)*

The preparation of compound **3** was carried out in an identical manner to compound **1**. Ph_2SnCl_2 (0.15 g, 0.43 mmol) and ligand salt (0.21 g 0.87 mmol). M.p. 150 °C; FT-IR (KBr): ν = 995 (C-S), 1482 (C-N), 449 (Sn-S), 543 (Sn-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 4.03 (t, $H_{3,3'}$, 8 H, $^3J_{H\text{-}H}$ = 10 Hz), 3.77 (t, $H_{2,2'}$, 8 H, $^3J_{H\text{-}H}$ = 10 Hz), 7.93 (H_O, SnC_6H_5, 4 H), 7.42 (m, $H_{m,p}$, SnC_6H_5, 6 H) ppm; ^{13}C NMR (500 MHz, DMSO): δ = 196.7 (C-1), 65.3 (C-3,3′), 51.7 (C-2,2′), 150 (C_α), 133 (C_β), 128.5 (C_δ), 128.2 (C_γ) ppm; ^{119}Sn NMR (500 MHz, DMSO): δ = −225 ppm; MS: *m/z* (70 eV) = 520 $(C_{16}H_{21}N_2O_2S_4Sn)^+$, 443 $(C_{10}H_{16}N_2O_2S_4Sn)^+$, 358 $(C_{11}H_{13}NOS_2Sn)^+$, 162 $(C_5H_8NOS_2)^+$, 86 $(C_4H_8NO)^+$, 44 $(C_2H_4O)^+$.

*Synthesis of dibenzyltin(IV)-bis(morpholine-1-carbodithioate) (**4**)*

The preparation of compound **4** was carried out in an identical manner to compound **1**. Bz_2SnCl_2 (0.12 g, 0.32 mmol) and ligand salt (0.16 g 0.64 mmol). M.p. 140 °C ; FT-IR (KBr): ν = 997.3 (C-S), 1474 (C-N), 417 (Sn-S), 544 (Sn-C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO): δ = 3.86 (t, $H_{3,3'}$, 8 H, $^3J_{H\text{-}H}$ = 10 Hz), 3.58 (t, $H_{2,2'}$, 8 H, $^3J_{H\text{-}H}$ = 10 Hz), 3.29 (s, H_α , 4 H), 7.15 (H_O, $SnCH_2C_6H_5$), 7.02 (m, $H_{m,p}$, Sn-$CH_2C_6H_5$) ppm; ^{13}CNMR (500 MHz, DMSO): δ = 199 (C-1), 65.3 (C-3,3′), 51 (C-2,2′), 30.6 (C_α) 139.3 (C_β), 128.7 (C_γ), 127.3(C_ε), 124.5 (C_δ) ppm; ^{119}Sn NMR (500 MHz, DMSO): δ = −484 ppm. MS: *m/z* (70 eV) = 372 $(C_{12}H_{15}NOS_2Sn)^+$, 301 $(C_{14}H_{14}Sn)^+$, 162 $(C_5H_8NOS_2)^+$, 91 $(C_7H_7\ )^+$, 77 $(C_6H_5)^+$.

In Vitro Studies

Preparation of drug solutions

Stock solutions of the studied compounds were prepared in DMSO (Sigma Aldrich) at a concentration of 1000 μg ml^{-1}, sterilized by filtration through Millipore filter (0.22 μm) before use, and diluted by cell culture medium to various working concentrations. DMSO was used owing to solubility problems. Nutrient medium was RPMI-1640 (Gibco BRL, UK) supplemented with 10% fetal bovine serum (FBS; Gibco BRL). MTT was dissolved (5 mg ml^{-1}) in phosphate buffered saline (PBS; pH 7.2) and filtered through Millipore filter (0.22 μm) before use.

HeLa cells were split using 0.25% TRED (trypsin–ethylenediaminetetraacetic acid; Gibco BRL) medium prior to reaching 80% confluence.

Cell line and cell culture

HeLa (human cervix carcinoma) cells (NCBI C115, National Cell Bank of Iran) and K562 (human myelogenous leukemia) cells (NCBI C122, National Cell Bank of Iran) were obtained from the Pasteur Institute of Iran. Cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco BRL) 2 mM L-glutamine (Gibco BRL) and antibiotics, including streptomycin (100 μg ml^{-1}) and penicillin (100 IU ml^{-1}) (Sigma, USA) and incubated at 37 °C in a humidified 5% CO_2 atmosphere.

HeLa cells were cultured as monolayers in the completed RPMI 1640 medium, while K562 cells were maintained as suspension culture. The cells were grown at 37 °C in 5% CO_2 and humidified air atmosphere. Peripheral blood mononuclear cells (PBMC) were separated from whole heparinized blood from a healthy volunteer by density gradient centrifugation using Lymphoprep (Nycomed, Oslo, Norway). Isolated cells, washed three times with PBS, then were counted and resuspended in nutrient medium. Cells were counted by Neubauer slide and Trypan blue dye exclusion method.

Trypan blue exclusion

The loss of membrane integrity, as a morphological characteristic for cell death, was assayed by Trypan blue dye exclusion.[33] Live cells were estimated using a hematocytometer and phase-contrast microscopy.

Cytotoxicity assay

HeLa cells were seeded (5000 cells per well) into 96 -well flat-bottom microtiter plates and incubated for 4 h prior to the addition of five different concentrations of the filtered studied compounds. Final concentrations achieved in treated wells were 0.001, 0.01, 0.1, 1 and 10 μg ml^{-1}. Each concentration was tested in quadruplicate on each cell line.

The final concentrations (<0.1%) of DMSO were non-toxic to the cells. Only complete medium was added to the cells in the control wells. The studied compounds were added to a suspension of leukemia K562 cells (10 000 cells per well) 4 h after cell seeding, in the same final concentrations applied to HeLa cells. Each assay included a blank containing complete medium without cells. PBMC were seeded (200 000 cells per well) in complete medium enriched with (2.5 μg ml^{-1}) phytohemagglutinin (Sigma) in 96-well microtiter plates, and 4 h later the compounds were added to the wells, in quadruplicate, to five final concentrations, except to the control wells, where enriched cell culture medium only was added to the cells. The incubation time was 72 h, during which period the control cells showed exponential growth.

Cytotoxicity experiments

Cell survival was determined by MTT test according to the method of Mosmann[34] and modified by Ohno and Abe,[35] which measures the reduction of the yellow tetrazolium salt MTT (Sigma) to a purple formazan crystal, mainly by activity of the mitochondrial enzymes cytochrome oxidase and succinate dehydrogenase. Briefly, cells were incubated for 72 h and then 20 μl of MTT solution (5 mg ml^{-1}) in PBS (1/10 of total volume in a well) was added to wells. Samples were incubated for a further 4 h at 37 °C in a humidified atmosphere with 5% CO2 (Fig. 2). Supernatants were removed and 100 μl DMSO was added to the plate as a solvent to each well. The plate was shaken for 15 min by a shaker incubator in order to dissolve the formazan crystals.

The optical density (OD) value was defined as the absorbance of each individual well, minus the blank value (blank is the mean OD of the control cells). Finally, the absorbance at 570 nm (test wavelength) and with a reference filter of 630 nm was measured using an ELISA microplate reader (Stat Fax-2100, USA). All experiments were performed three times and the percentage of cytotoxicity was calculated according to following formula:

$$\%\ \text{cytotoxicity} = 1 - \frac{\text{mean absorbance of toxicant-treated cells}}{\text{mean absorbance of negative control}} \times 100$$

$$\%\ \text{viability} = 100 - \%\ \text{cytotoxicity}$$

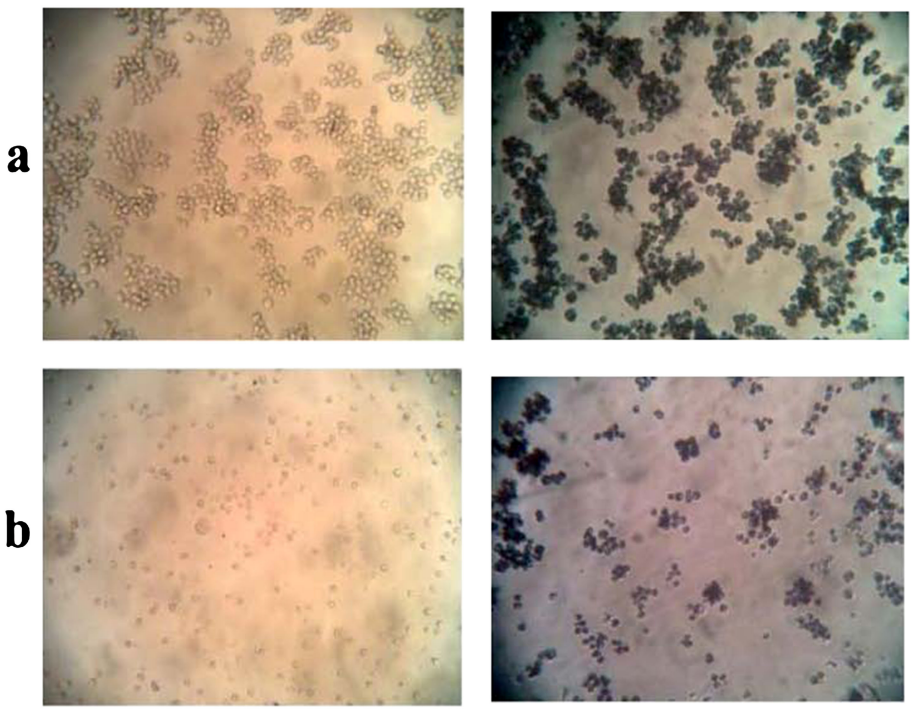


Figure 2. Photographed under an inverted microscope. Untreated K562 cells (control) before and after MTT assay (a) and cells treated for 72 h with 1 μg ml^{-1} compound **1** before and after MTT assay (b).

Data Analysis

After subtracting the solvent toxicity, the concentration giving 50% inhibition (IC_{50}) was determined for the test samples by non-linear regression analysis of curves. Graph Pad Prism version 4.00 was used to calculate IC_{50}. Mean difference among groups was calculated by paired *t*-test, one-way and repeated-measures analysis of variance (ANOVA) ($p < 0.05$).

Conclusions and Outlook

Four diorganotin(IV) complexes containing dithiocarboxylate ligand have been synthesized and structurally characterized by different analytical techniques. The results indicate the diverse structural motifs for these compounds (**1–4**) depending upon the mode of coordination of ligand as well as the presence or absence of intermolecular S-Sn interactions. These compounds were tested *in vitro* against human tumor cell lines – HeLa and K562 – and stimulated normal immunocompetent cells: PBMC.

The studied organotin(IV) compounds presented high activity against the evaluated tumoral cell lines. Compound **4** (which contains the benzyl group) is the most active complex against K562 cell line, and compound **1** (which contains the methyl group) is highly active against HeLa cells. Future work, already in progress, will now focus on the improvement of the cytotoxic nature of compounds **1** and **4** and introduction of other different dithiocarboxylate ligands to improve the water solubility and cytotoxic activity of the complexes. Further studies on other cell lines will also be carried out.

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