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FULL PAPER

Preparation and *in vitro* investigations of triphenyl[ω-(tetrahydro-2*H*-pyran-2-yloxy)alkyl]tin(IV) compounds

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The reaction of $SnPh_3Li$ with $X(CH_2)_nO$–THP (THP = tetrahydro-2*H*-pyran-2-yl; n = 3, 4, 6, 8, 11; X = Cl, Br) afforded organotin(IV) compounds with the general formula $Ph_3Sn(CH_2)_nO$–THP (**1**–**5**). The tetraorganotin(IV) compounds were characterized using multinuclear NMR and infrared spectroscopies and high-resolution mass spectrometry. Anticancer activity of the synthesized compounds was tested *in vitro* against the A2780 (ovarian), A549 (lung), HeLa (adenocarcinoma) and SW480 (colon) tumour cell lines with SRB assay. The *in vitro* investigations revealed that when a shorter chain was present a higher activity was achieved; however compounds **1**–**5** were found to be less active than cisplatin. In addition, the most active compound, **1**, enters A2780 cells and causes apoptosis by triggering both intrinsic and extrinsic caspase pathways.

KEYWORDS

apoptosis, caspase, cytotoxicity, organotin(IV) compounds

1 | INTRODUCTION

Platinum-based compounds have been in focus for many years as potential antitumor agents since the discovery of cisplatin in the 1960s.[1–8] In the meantime, in the same field research is also directed to the development of non-platinum metal-based compounds (e.g. Ru(II), Ti(IV), Au(III), Ga(III), Sn(IV))[9–13] because they can have different targets in cells and might exhibit mechanisms of action in cancer cells other than those of platinum-based ones. Tin(IV) compounds are interesting because of well-known multiple applications: industrial and agricultural applications as well as biological (biocides, antimicrobials and potential antiviral and anticancer agents).[14–17] In the last few decades the anti-tumour activity of organotin(IV) compounds has been quite well investigated.[9,18,19] Some of the organotin(IV) compounds, e.g. tricyclohexyltin(IV) derivatives, are capable of overcoming the multidrug resistance noticed for other commercial metal-containing drugs,[20] or are more selective towards tumour cells than cisplatin, such as $[NHEt_3][SnPh_3ClL]$ compounds (L = *N*-phthaloylglycinato, *N*-phthaloyl-L-alaninato and 1,2,4-benzenetricarboxylato-1,2-anhydride).[21] Mostly, organotin(IV) compounds induce apoptosis in various cancer cell lines.[17]

Various mono- and polynuclear organotin(IV) compounds comprised of di- or triorganyltin(IV) (organyl = methyl, phenyl, etc.) and carboxylato or thiolato ligands showed high *in vitro* anticancer activity.[22–28] Generally, the most active are found to be triphenyltin(IV) compounds (mostly in nM concentration), much more active than cisplatin. On the other hand, less active are found to be tin(IV) compounds bearing Me groups, as well being less active than cisplatin. However, in the literature are described some compounds which do not follow such structure–activity relationship.

Herein is reported the synthesis and structural characterization of triphenyltin(IV) compounds containing ω-(tetrahydro-2*H*-pyran-2-yloxy)alkyl moieties (alkyl = *n*-propyl (**1**), *n*-butyl (**2**), *n*-hexyl (**3**), *n*-octyl (**4**), *n*-undecyl (**5**)). Furthermore, the cytotoxic activity of **1**–**5** against ovarian A2780, lung A549, adenocarcinoma HeLa and colon SW480 tumour cell lines was investigated. Accumulation in cells, induction of apoptosis and caspase activation of the most active compound, **1**, were tested against the A2780 cell line.

*Dedicated to Prof. Dr Dirk Steinborn on the occasion of his 70th birthday.

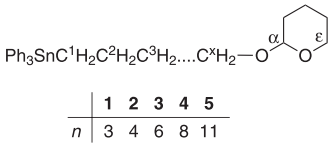
2 | EXPERIMENTAL

2.1 | Materials and methods

Synthesis of **1–5** was performed under an atmosphere of dry argon using standard Schlenk techniques. Before use, solvents were distilled from the appropriate drying agents and degassed. NMR spectra were recorded on a Varian INOVA UNITY 500 NMR spectrometer (11.74 T, ^{1}H NMR (499.75 MHz), internal standard solvent; ^{13}C NMR (125.7 MHz), internal standard solvent; ^{119}Sn NMR (186.3 MHz), external standard $(CH_3)_4Sn$ (0.0 ppm)) and on a Varian VNMRS 400 NMR spectrometer (9.35 T, ^{1}H NMR (399.96 MHz), internal standard solvent; ^{13}C NMR (100.6 MHz), internal standard solvent; ^{119}Sn NMR (149.1 MHz), external standard $(CH_3)_4Sn$). Electrospray high-resolution mass spectra (HR-ESI-MS) were obtained in positive- and negative-ion modes on a Bruker Apex III (FTR-ICR) mass spectrometer. Infrared (IR) spectra were measured from 4000 to 250 cm^{-1} on a Bruker Tensor 27 FT-IR spectrometer with diamond ATR. Commercially available reagents Ph_3SnCl and 3,4-dihydro-2*H*-pyran (THP) were purchased from Acros and used without further purification, while 2-[(3-chloropropyl)oxy]tetrahydropyrane, 2-[(4-chlorobutyl)oxy]tetrahydropyrane, 2-[(6-chlorohexyl)-oxy]tetrahydropyrane, 2-[(8-bromoctyl)oxy]tetrahydropyrene and 2-[(11-bromoundecyl)oxy]tetrahydropyrane were synthesized using procedures from the literature.[29]

2.2 | General procedure for synthesis of triphenyl[ω-(tetrahydro-2*H*-pyrane-2-yl-oxy)alkyl]stannanes (1–5)

The synthesis was carried out following known literature procedures.[30–33] Elemental lithium (2.5 eq, 180 mg) was added to THF (20 ml) and refluxed for 30 min. Triphenyltin chloride (1 equation 4.2 g) was added to the reaction mixture, which turned to dark green, and refluxed for 6 h with vigorous stirring. After completion of the reaction, the mixture was filtered through glass wool. The particular 2-[(ω-halogenalkyl) oxy]tetrahydropyrane (0.5 eq.) was added via a syringe and the reaction mixture was stirred overnight at room temperature. To complete the conversion, necessary for **3–5**, the reaction mixture was heated for 6 h under reflux. The dark green solution was quenched with HCl (2.5 ml), the reaction mixture turning orange. To complete hydrolysis, the solution was stirred for 30 min at room temperature. The solvent was distilled off via rotary evaporation. Colum chromatography yielded pure compounds. Figure 1 shows the numeration of C atoms.



	1	2	3	4	5
n	3	4	6	8	11

FIGURE 1 Numeration of C atoms.

Triphenyl[3-(tetrahydro-2*H*-pyran-2-yloxy)propyl]-stannane (1). Purification: *n*-hexane/ethyl acetate/acetic acid (7/3/0.01). Yield 1.536 g (68%); pale yellow, highly viscous oil, odourless. HR-ESI-MS: *m/z* for $[C_{26}H_{30}O_2NaSn]^+$ calcd: 517.1160, found: 517.1160. IR (ATR, cm^{-1}): 2995 (w), 2912 (w), 1436 (w), 1407 (w), 1309 (w), 1042 (vs), 954 (m), 697 (m), 668 (w), 380 (m), 331 (s). ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 1.49–1.90 (m, 8H; $C^{\beta,\gamma,\delta,1}H_2$), 2.09–2.19 (m, 2H; $C^{2}H_2$), 3.44–3.53 and 3.80–3.91 (m, 4H; $C^{\varepsilon,3}H_2$), 4.52–4.56 (m, 1H; $C^{\alpha}H$), 7.40–7.45 (m, 9H; Ar-H_m and Ar-H_p), 7.60–7.66 (m, 6H; Ar-H_o). ^{13}C NMR ($CDCl_3$, 101 MHz, δ, ppm): 7.2 (C^{1}, ^{1}J(^{119}Sn,C) = 396.7 Hz), 19.7 (C^{γ}), 25.5 (C^{δ}), 26.7 (C^{2}, ^{2}J(^{119}Sn,C) = 20.0 Hz), 30.7 (C^{β}), 62.4 (C^{ε}), 70.4 (C^{3}, ^{3}J(^{119}Sn,C) = 72.1 Hz), 99.0 (C^{α}), 128.5 (C^{x}, ^{3}J(^{119}Sn,C) = 48.9 Hz), 128.9 (C_p, ^{4}J(^{119}Sn,C) = 11.0 Hz), 137.1 (C_o, ^{2}J(^{119}Sn,C) = 36.1 Hz), 138.9 (C_i, ^{1}J(^{119}Sn,C) = 489.4 Hz). ^{119}Sn NMR ($CDCl_3$, 149 MHz, δ, ppm) = −99.0 (s, Ph_3Sn).

Triphenyl[4-(tetrahydro-2*H*-pyran-2-yloxy)butyl]-stannane (2). Purification: *n*-hexane/ethyl acetate/acetic acid (8/2/0.01). Yield 0.375 g (18%); pale yellow, highly viscous oil, odourless. HR-ESI-MS: *m/z* for $[C_{27}H_{32}O_2NaSn]^+$ calcd: 531.1316, found: 531.1316. IR (ATR, cm^{-1}): 3445 (b), 2997 (w), 2914 (w), 1661 (b), 1436 (w), 1407 (w), 1312 (w), 1020 (vs), 953 (m), 698 (m), 668 (w), 382 (m), 332(s). ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 1.49–1.93 (m, 12H; $C^{\beta,\gamma,\delta,1-3}H_2$), 3.39–3.46 and 3.74–3.79 (m, 2H; $C^{\varepsilon}H_2$), 3.47–3.53 and 3.80–3.87 (m, 2H; $C^{4}H_2$), 4.55–4.59 (m, 1H; $C^{\alpha}H$), 7.38–7.44 (m, 9H; Ar-H_m and Ar-H_p), 7.57–7.63 (m, 6H; Ar-H_o). ^{13}C NMR ($CDCl_3$, 101 MHz, δ, ppm): 10.8 (C^{1}, ^{1}J(^{119}Sn,C) = 394.5 Hz), 19.6 (C^{γ}), 23.4 (C^{2}, ^{2}J(^{119}Sn, C) = 20.9 Hz), 25.6 (C^{δ}), 30.7 (C^{β}), 34.1 (C^{3}, ^{3}J(^{119}Sn, C) = 64.1 Hz), 62.1 (C^{ε}), 66.7 (C^{4}), 98.7 (C^{α}), 128.5 (C^{x}, ^{3}J(^{119}Sn,C) = 48.9 Hz), 128.9 (C_p, ^{4}J(^{119}Sn,C) = 11.0 Hz), 137.1 (C_o, ^{2}J(^{119}Sn,C) = 36.1 Hz), 138.9 (C_i, ^{1}J(^{119}Sn, C) = 489.4 Hz). ^{119}Sn NMR ($CDCl_3$, 149 MHz, δ, ppm): −100.1 (s, Ph_3Sn).

Triphenyl[6-(tetrahydro-2*H*-pyran-2-yloxy)hexyl]-stannane (3). Purification: *n*-hexane/ethyl acetate/acetic acid (9/1/0.01). Yield 1.166 g (47%); pale yellow, highly viscous oil, odourless. HR-ESI-MS: *m/z* for $[C_{29}H_{36}O_2NaSn]^+$ calcd: 559.1629, found: 559.1631. IR (ATR, cm^{-1}): 2996 (w), 2912 (w), 1435 (w), 1405 (w), 1311 (w), 1042 (vs), 952 (m), 700 (m), 668 (w), 382 (m), 331 (s). ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 1.33–1.91 (m, 16H; $C^{\beta,\gamma,\delta,1-5}H_2$), 3.38–3.42 and 3.70–3.77 (m, 2H; $C^{\varepsilon}H_2$), 3.50–3.57 and 3.87–3.94 (m, 2H; $C^{6}H_2$), 4.58–4.61 (m, 1H; $C^{\alpha}H$), 7.39–7.45 (m, 9H; Ar-H_m and Ar-H_p), 7.57–7.63 (m, 6H; Ar-H_o). ^{13}C NMR ($CDCl_3$, 101 MHz, δ, ppm): 11.1 (C^{1}, ^{1}J(^{119}Sn,C) = 396.8 Hz), 19.8 (C^{γ}), 25.6 (C^{δ}), 25.8 (C^{4}), 26.6 (C^{2}, ^{2}J(^{119}Sn,C) = 22.1 Hz), 29.7 (C^{5}), 30.8 (C^{β}), 34.1 (C^{3}, ^{3}J(^{119}Sn,C) = 62.2 Hz), 62.3 (C^{ε}), 66.6 (C^{6}), 99.8 (C^{α}), 128.5 (C^{x}, ^{3}J(^{119}Sn,C) = 48.3 Hz), 128.8 (C_p, ^{4}J(^{119}Sn, C) = 10.9 Hz), 137.1 (C_o, ^{2}J(^{119}Sn,C) = 35.1 Hz), 139.1

(C_i, $^1J(^{119}Sn,C) = 479.4$ Hz). ^{119}Sn NMR ($CDCl_3$, 149 MHz, δ, ppm): −99.7 (s, Ph_3Sn).

Triphenyl[8-(tetrahydro-2*H*-pyran-2-yloxy)octyl]-stannane (4). Purification: *n*-hexane/ethyl acetate/acetic acid (9/1/0.01). Yield 0.704 g (32%); pale yellow, highly viscous oil, odourless. HR-ESI-MS: *m/z* for $[C_{31}H_{40}O_2NaSn]^+$ calcd: 587.1942, found: 587.1949. IR (ATR, cm^{-1}): 2925 (w), 2850 (w), 1428 (m), 1117 (w), 1074 (m), 1023 (s), 904 (w), 868 (w), 813 (w), 727 (s), 698 (vs), 658 (w), 445 (m), 380 (w), 332 (w), 261 (s). ^{1}H NMR ($CDCl_3$, 500 MHz, δ, ppm): 1.25–1.91 (m, 20H; $C^{\beta,\gamma,\delta,1-7}H_2$), 3.37–3.44 and 3.73–3.79 (m, 2H; $C^{\varepsilon}H_2$), 3.50–3.57 and 3.88–3.94 (m, 2H; $C^{8}H_2$), 4.62 (br, 1H; $C^{\alpha}H$), 7.40 (br, 9H; Ar-H_m and Ar-H_p), 7.56–7.62 (m, 6H; Ar-H_o). ^{13}C NMR ($CDCl_3$, 126 MHz, δ, ppm): 11.1 (C^1, $^1J(^{119}Sn,C) = 398.5$ Hz), 19.6 (C^{γ}), 25.5 (C^{δ}), 26.1 (C^4), 26.5 (C^2, $^2J(^{119}Sn,C) = 23.3$ Hz), 28.9, 29.3 (C^5 and C^6), 29.7 (C^7), 30.8 (C^{β}), 34.1 (C^3, $^3J(^{119}Sn,C) = 61.8$ Hz), 62.2 (C^{ε}), 67.6 (C^8), 98.7 (C^{α}), 128.4 (C^x, $^3J(^{119}Sn,C) = 48.1$ Hz), 128.7 (C_p, $^4J(^{119}Sn,C) = 11.1$ Hz), 137.0 (C_o, $^2J(^{119}Sn,C) = 35.8$ Hz), 139.1 (C_i, $^1J(^{119}Sn,C) = 479.7$ Hz). ^{119}Sn NMR ($CDCl_3$, 186 MHz, δ, ppm): −99.5 (s, Ph_3Sn).

Triphenyl[11-(tetrahydro-2*H*-pyran-2-yloxy)undecyl]-stannane (5). Purification: *n*-hexane/ethyl acetate/acetic acid (7/3/0.01). Yield 1.020 g (57%); pale yellow, highly viscous oil, odourless. HR-ESI-MS: *m/z* for $[C_{34}H_{46}O_2NaSn]^+$ calcd: 629.2412, found: 629.2413. IR (ATR, cm^{-1}): 3445 (b), 3000 (w), 2915 (w), 1661 (b), 1434 (w), 1405 (w), 1310 (w), 1023 (vs), 952 (m), 698 (m), 670 (w), 381 (m), 332 (s). ^{1}H NMR ($CDCl_3$, 400 MHz, δ, ppm): 1.19–1.93 (m, 31H; $C^{\beta,\gamma,\delta,1-10}H_2$), 3.39–3.46 and 3.74–3.82 (m, 2H; $C^{\varepsilon}H_2$), 3.50–3.58 and 3.88–3.96 (m, 2H; $C^{11}H_2$), 4.60–4.65 (m, 1H; $C^{\alpha}H$), 7.37–7.43 (m, 9H; Ar-H_m and Ar-H_p), 7.55–7.62 (m, 6H; Ar-H_o). ^{13}C NMR ($CDCl_3$, 101 MHz, δ, ppm): 11.2 (C^1, $^1J(^{119}Sn,C) = 398.0$ Hz), 19.7 (C^{γ}), 25.6 (C^{δ}), 26.3 (C^4), 26.6 (C^2, $^2J(^{119}Sn,C) = 22.3$ Hz), 29.1, 29.5, 29.5, 29.6, 29.6, (C^5–C^9), 29.9 (C^{10}), 30.8 (C^{β}), 34.3 (C^3, $^3J(^{119}Sn,C) = 60.1$ Hz), 62.3 (C^{ε}), 67.7 (C^{11}), 98.9 (C^{α}), 128.4 (C^x, $^3J(^{119}Sn,C) = 48.0$ Hz), 128.8 (C_p, $^4J(^{119}Sn,C) = 11.0$ Hz), 137.1 (C_o, $^2J(^{119}Sn,C) = 35.1$ Hz), 139.2 (C_i, $^1J(^{119}Sn,C) = 480.3$ Hz). ^{119}Sn NMR ($CDCl_3$, 149 MHz, δ, ppm): −99.6 (s, Ph_3Sn).

2.3 | *In vitro* studies

RPMI-1640, phosphate-buffered saline (PBS), penicillin/streptomycin, 10× Dulbecco's PBS without Ca^{2+} and Mg^{2+}, trypsin–EDTA 0.05%/0.02% (PAA Laboratories GmbH), proteinase K 20 mg ml^{-1} in PBS, RNAse A (Roche Diagnostics GmbH) and specific caspase substrates (2, Ac-VDVAD-pNA; 3, Ac-DEVD-pNA; 8, Ac-IETDpNA; 9, Ac-LEHD-pNA; Axxora, Loerrach, Germany) were used. Human tumour cell lines A2780 (ovarian), A549 (lung), HeLa (adenocarcinoma) and SW480 (colon) were routinely maintained in RPMI-1640 medium supplemented with 10% foetal calf serum and 1% antibiotics (culture medium) at 37° C in a humidified atmosphere with 5% CO_2. Stock solutions of investigated compounds (20 mM) were freshly prepared in dimethylsulfoxide and diluted to various working concentrations with medium.

2.3.1 | SRB assay

The viability of adherent viable cells was measured by SRB assay.[34,35] Cells were exposed to a wide range of doses of the compounds for 96 h and then fixed with 10% trichloroacetic acid for 2 h at 4°C. After fixation, cells were washed in distilled water, stained with 0.4% SRB solution for 30 min at room temperature, washed and dried overnight. The dye was dissolved in 10 mM Tris buffer and the absorbance was measured at 570 nm. IC_{50} values, defined as the concentrations of the compounds at which 50% cell inhibition occurs, were calculated using four-parameter logistic function and presented as the mean from three independent experiments.

2.3.2 | Inductively coupled plasma (ICP) MS analysis

A2780 cells (1.2×10^6) were seeded in a flask. After 24 h, cells were treated with IC_{50} dose of **1** (10 ml) for 24 h. Afterwards the cells were washed with PBS (4 ml) and trypsinized (1 ml). Cells were resuspended in PBS (4 ml) and centrifuged (5000 U min^{-1}, 5 min). Cell pallet was washed twice with PBS (1 ml). Lyophilized cells were analysed using ICP MS (Wessling Laboratorien GmbH, Oppin, Germany).

2.3.3 | Morphological analysis (acridine orange (AO)/ethidium bromide (EB) double staining)

Compound **1**- and cisplatin-induced cell death in A2780 ovarian tumour cells was determined using AO and EB double staining according to standard procedures and examined under a fluorescence microscope.[36] A2780 cells were seeded overnight on eight-chamber slides (6000 cells per chamber) in 400 µl of complete medium. The next day, cells were treated with $2 \times IC_{50}$ of the investigated compounds for 24 h. After this period, cells on the slides were stained with AO/EB mixture (3 µg ml^{-1} AO and 10 µg ml^{-1} EB in PBS), and visualized under a fluorescence microscope (Carl Zeiss).

2.3.4 | Caspase 2, 3, 8 and 9 enzyme activity assays

Activity of caspases 2, 3, 8 and 9 was measured using the caspase substrate cleavage assay. A2780 cells were seeded at a density of 1×10^6 per well in a six-well plate. After exposure to IC_{50} concentration of **1**, cells were sampled after 2 h for cleavage of caspases. Adherent cells were washed with cold PBS, collected with a cell scraper and suspended in cell lyses buffer (50 mM Hepes, pH = 7.4, 1% Triton X100, all from Sigma-Aldrich). After incubation for 10 min on ice and centrifugation, protein concentrations of the supernatants were measured according to a method of Bradford (Bio-Rad

Laboratories). Samples (50 μg protein extract) were incubated on a microplate at 37°C overnight in reaction buffer (50 mM Hepes, pH = 7.4, 0.1% CHAPS, 5 mM EGTA, 5% glycerol) containing 10 mM DTT (all from Sigma-Aldrich) and a specific substrate of caspases. Extinction of released *p*-nitroaniline was measured at 405 nm using a 96-well plate reader (Tecan Spectra) and activity of caspases 2, 3, 8 and 9 was evaluated by optical density ratio of treated/untreated samples.[13,21]

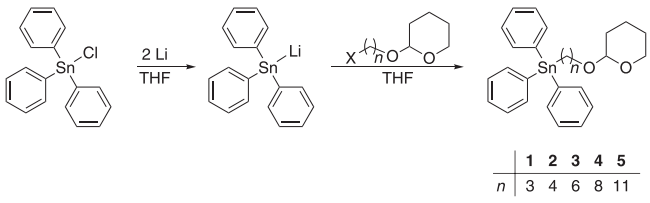
3 | RESULTS AND DISCUSSION

3.1 | Synthesis and characterization

Compounds **1**–**5** of the general formula $Ph_3Sn(CH_2)_nO$–THP (n = 3, 4, 6, 8, 11) have been synthesized in the reaction of Ph_3SnLi with THP-protected ω-halogen alkylalcohols (X $(CH_2)_nO$–THP; n = 3, 4, 6, 8, 11; X = Cl, Br; Scheme 1). Although compounds of this type could be synthesized in the reaction of Ph_3SnCl and α,ω-halogen(tetrahydro-2*H*-pyran-2-yloxy)alkane via Grignard reagents, additional deprotection and protection strategy is necessary for the synthesis of pure products in poor yields.[37] After preparative chromatography using the synthetic procedure presented herein, the compounds were isolated as colourless, highly viscous oils in yields of up to 67%.

HR-ESI-MS gave evidence for molecular composition of the organotin(IV) compounds **1**–**5**. For all compounds, $[M + Na]^+$ ions in positive mode are formed.

The constitution of compounds **1**–**5** was determined using multinuclear NMR spectroscopy (^1H, ^{13}C and ^{119}Sn). Selected data are presented in Table 1. As expected, chemical



SCHEME 1 Synthesis of $Ph_3Sn(CH_2)_nO$–THP (**1**–**5**).

shifts of the carbon atoms C^1–C^3 in ^{13}C NMR spectra and coupling constants of ^{119}Sn with C atom, observed in ^{13}C NMR spectra, remain unchanged for **2**–**5**. Except for **1**, because of a shorter alkyl spacer, the ether oxygen atom influences the mentioned chemical shifts and coupling constants. The neighbouring hydrogen and carbon atoms of those ether oxygen atoms show similar trend in chemical shifts in ^1H NMR and ^{13}C NMR spectra. Thus, CH_2 groups from pyranyl ring and alkyl chain separate in two distinguishable multiplets. Tertiary carbon atom from pyranyl ring shows a chemical shift in lower field. ^{119}Sn NMR spectra of **1**–**5** confirm that they are isolated Ph_3Sn(alkyl) compounds.[25,38]

3.2 | *In vitro* activity

The synthesized tetraorganotin(IV) compounds were investigated *in vitro* against four different human tumour cell lines.[35] Dose-dependent graphs are presented in Fig. 2 and IC_{50} values are presented in Table 2. Cisplatin is used for comparison.

A general trend can be observed for all cell lines when treated with compounds **1**–**5**. Namely, as the length of spacer between tin atom and THP moiety in $Ph_3Sn(CH_2)_nO$–THP (n = 3, 4, 6, 8, 11) increases, the activity decreases (IC_{50}: **1** ≈ **2** < **3** < **4** < **5**). Thus, the most active compounds are those with shorter chains without significant difference

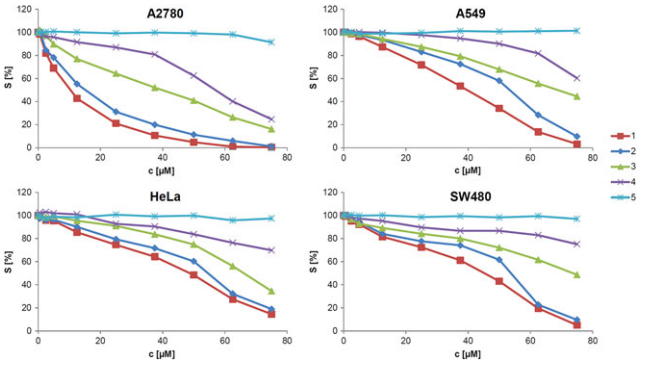


FIGURE 2 Survival graphs after 96 h of treatment for A2780, A549, HeLa and SW480 cells with organotin(IV) compounds **1**–**5** determined with SRB assay (SD < 10%, not shown).

TABLE 1 ^1H NMR, ^{13}C NMR and ^{119}Sn NMR spectroscopic parameters (δ, ppm; J, Hz) for **1**–**5**

	1	2	3	4	5
$\delta_H{}^{\alpha}$	4.52–4.56	4.55–4.59	4.58–4.61	4.62	4.60–4.65
$\delta_C{}^1$	7.2	10.8	11.1	11.1	11.2
$^1J(^{119}Sn,C)$	396.7	394.5	396.8	398.5	398.0
$\delta_C{}^2$	26.7	23.4	26.6	26.5	26.6
$^2J(^{119}Sn,C)$	20.0	20.9	22.1	23.3	22.3
$\delta_C{}^3$	70.4	34.1	34.1	34.1	34.3
$^3J(^{119}Sn,C)$	72.1	64.1	62.2	61.8	60.1
$\delta_C{}^{e}$	62.4	62.1	62.3	62.2	62.3
$\delta_C{}^{x}$	see C^3	66.7	66.6	67.6	67.7
$\delta_{S\ n}$	−99.0	−100.1	−99.7	−99.5	−99.6

TABLE 2 IC_{50} values (μM) of **1**–**5** against A2780, A549, HeLa and SW480 cell lines (96 h)

Compound	A2780	A549	HeLa	SW480
1	9.27 ± 1.13	37.72 ± 4.97	45.77 ± 1.27	42.94 ± 1.01
2	13.53 ± 2.29	50.84 ± 7.90	52.86 ± 1.41	52.82 ± 8.71
3	35.44 ± 1.52	51.77 ± 2.30	66.00 ± 1.73	72.20 ± 1.06
4	57.55 ± 9.13	>100	>100	>100
5	>100	>100	>100	>100
Cisplatin	0.55 ± 0.33	1.54 ± 0.28	4.60 ± 0.34	3.22 ± 0.24

between propyl and butyl moieties. On the other hand, discrimination between tumour cells upon action of **1**–**5** is more than obvious. The highest activity of investigated compounds is observed against ovarian A2780 cells, followed by lung A253, adenocarcinoma HeLa and colon SW480 cells. Furthermore, results reported herein are contrary to findings for $[PtCl_2\{NH_2CH_2CH_2NH(CH_2)_nO–THP\}]$ or $[PtCl_2(NH_2CH_2CH\{(CH_2)_nO–THP\}CH_2NH_2)]$ complexes (n = 3, 4, 6, 8, 11), where the most active complexes were found to be those with longer lipophilic spacers.[29,39] However, the activity of the organotin compounds is lower than that of cisplatin (Table 2). Compound **1** as well as the A2780 cell line were selected for further investigations because of the greatest activity of this compound against those cells.

3.3 | Organotin(IV) compound 1 enters A2780 cells and causes apoptosis

To determine if compound **1** accumulates in the cells, A2780 cells were treated with IC_{50} dose of **1** for 24 h and tin uptake was assessed using ICP MS analysis. It is found that **1** is present in high concentration, 2500 ppm, in the A2870 cell line. In order to investigate if apoptosis is involved in A2780 cell death upon treatment with **1**, at first double staining AO/EB assay was performed and cells were analysed by fluorescence microscopy (Fig. 3A).

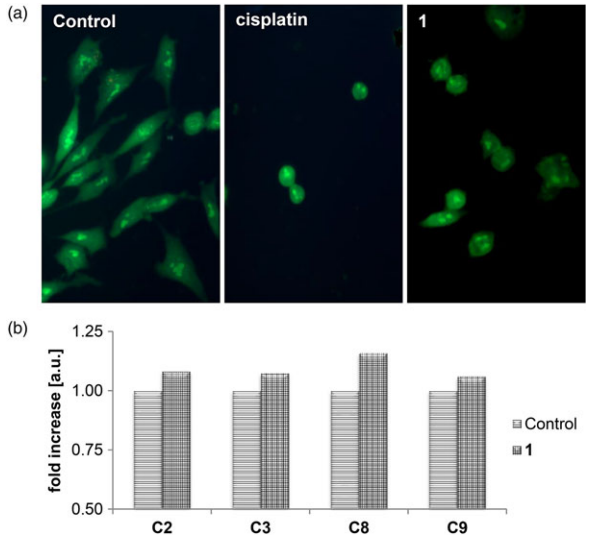


FIGURE 3 (a) AO/EB double-stained A2780 cells after treatment with **1** or cisplatin. (b) Caspase 2, 3, 8 and 9 activation after 2 h of treatment.

The supravital dye AO enters cells independently from disruption of membrane integrity and might fluoresce from green to orange. On the other hand, EB can only react with its cellular targets upon membrane disruptions (red fluorescence). Non-treated A2780 cells (control) as well as cells treated with **1** or cisplatin were allowed to grow for 24 h and afterwards investigated using fluorescence microscopy. As expected, control cells show normal morphology of A2780 cells, thus flattened vital cells, along with marginal number of apoptotic cells. Obviously morphological changes occur upon treatment of A2780 cells with **1** or cisplatin (Fig. 3A). In both cases cells do not show any sign of neither secondary necrosis nor necrosis. Cell morphological changes are seen as rounding of the cells as chromatin condensations which indicate that **1**, as well as cisplatin, causes apoptosis in A2780 cells.

In order to understand which mechanism is involved in triggering apoptosis, as seen in AO/EB double staining assay, it was analyzed whether caspases are involved as downstream effectors in induced cell death. Thus, the activities of upstream caspases 2, 8 and 9 as well as downstream caspase 3 were analysed in the present study. A2780 cells treated with compound **1** for 2 h show activation of all investigated caspases (Fig. 3B). Based on these results, organotin(IV) compound **1** triggers apoptosis by both internal (intrinsic or mitochondrial pathway, caspase 9 pathway) and external signals (extrinsic or death receptor pathway).

4 | CONCLUSIONS

Five ω-TPH-functionalized alkyltriphenyltin(IV) compounds were prepared and characterized. The cytotoxic activity of these compounds has been tested against ovarian A2780, lung A549, adenocarcinoma HeLa and colon SW480 tumour cell lines. Cytotoxic results pointed to a structure–activity relationship: for shorter alkyl chains, a higher activity is achieved. Against the investigated cell lines all compounds are found to be less active than cisplatin. The most active compound, **1**, enters A2780 cells and causes apoptosis by activation of both intrinsic and extrinsic caspase pathways.

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