



# Cytotoxic effect, antitumour activity and toxicity of organotin derivatives with ortho- or para-hydroxy-benzoic acids

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Received: 7 September 2017 / Accepted: 13 January 2018 / Published online: 2 February 2018  
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## Abstract

The cytotoxic and antitumour activities of five organotin complexes (**1–5**) with *o*-hydroxy-benzoic or *p*-hydroxy-benzoic acids were evaluated in a series of in vitro and in vivo experiments. All complexes exhibited strong cytotoxic activity against all cancer cells lines, whereas complexes **1**, **2** and **4** induced apoptosis at significantly lower doses than complexes **3** and **5**. Human cancer cells treated with increasing concentrations of complexes **1**, **2** and **4** gradually lost their ability to form colonies. Only complexes **1** and **2** inhibited colony formation efficiency in rat leiomyosarcoma cells. Histopathology of liver and kidney showed mild damage and lung oedema after a single injection of 10 mg/kg body wt of complex **1** to Wistar rats. At higher doses (100 mg/kg body wt) brain stem oedema was observed. Daily administration of tumour-bearing Wistar rats (leiomyosarcoma) with 1 mg/kg body wt of complex **1** until death reduced the mean tumour growth rate by more than 3× fold and prolonged mean survival time by 120%. These findings indicate that the organotin complexes with ortho- or para-hydroxy-benzoic acids possess potent cytotoxic and antitumour activity and they could be used as potential chemotherapeutic agents.

**Keywords** Organotin complexes · Hydroxy benzoic acid · Cytotoxicity · Anticancer · In vivo · Toxicity

## Introduction

Cisplatin is one of the most potent anticancer agent used alone or in combination with chemotherapy agents to treat various human tumours (Miller et al. 2010). However, the serious side effects (mainly nephrotoxicity) exerted by cisplatin (Miller et al. 2010) in conjunction with the increased

tumour resistance (Koberle et al. 2010) lead to the development of platinum and non-platinum metal complexes with similar anticancer activity (Garbutcheon-Singh et al. 2011). Especial interest was invested on organotin derivatives characterised by potent cytotoxic and anticancer activity (Arjmand et al. 2014; Amir et al. 2014; Muhammad and Guo 2014). Organotin derivatives can cause direct DNA damage (Liu et al. 2006) acting through different mechanisms than cisplatin, e.g., the rise of cytosolic Ca<sup>2+</sup> which leads the cells to apoptosis (Aw et al. 1990) or the inhibition of essential macromolecules synthesis and the mitochondrial metabolism (Alama et al. 2009). Moreover, the cytotoxicity exerted by some organotin complexes is correlated with the inhibition of peroxidation of linoleic acid induced by lipoxygenase (Abdellah et al. 2009).

Organotin derivatives show potent cytotoxic properties that can be even 200 times higher of cisplatin (Verginadis et al. 2011). Triphenyltin(VI) complexes of non-steroidal anti-inflammatory drugs were found to be highly cytotoxic against the cancer cell lines MCF-7 (breast), T-24 (urinary bladder) and A549 (lung) (Dokorou et al. 2011), while triphenyltin 2-(triphenyltinmercapto) nicotinate exhibited an

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IC<sub>50</sub> as low as 5.5 nM against leiomyosarcoma (LMS) cells (Verginadis et al. 2011). Moreover, organotin(IV) derivatives of L-cysteine show cytotoxic activity against six human cancer cells lines: leukaemia (HL60), liver (Bel7402), stomach (BGC823), cervix epithelial (HeLa), nasopharyngeal (KB) and lung (PG) (Chasapis et al. 2004). Hence, these compounds exert cytotoxic activity against cisplatin-sensitive and cisplatin-resistant cell lines. In vivo experiments in tumour bearing animals (LMS) with organotin metal complexes prolonged the mean survival time (MST) of the animals (Verginadis et al. 2011; Metsios et al. 2012) and inhibited tumour growth (Verginadis et al. 2011) with minor side effects (mainly in the kidney) (Verginadis et al. 2011; Metsios et al. 2012) or liver damage (Metsios et al. 2012).

The aim of this study was to examine the cytotoxic effects of organotin compounds with ortho- and para-hydroxybenzoic acid on malignant cell lines and to evaluate their toxic effects as well as their antitumour properties in a series of in vitro and in vivo experiments.

## Materials and methods

### Cell lines and culture conditions

Human cell lines MCF-7 (breast adenocarcinoma - ATCC<sup>®</sup>, HTB-22<sup>™</sup>), HeLa (cervical cancer—ATCC<sup>®</sup>, CCL-2<sup>™</sup>), U2-OS (human osteosarcoma—ATCC<sup>®</sup>, HTB-96<sup>™</sup>) and MRC-5 (normal foetal lung fibroblasts - ATCC<sup>®</sup>, CCL-171<sup>™</sup>) as well as cells (LMS) isolated from tumours histological identified as LMS in Wistar rats (Avdikos et al. 2007) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 1% penicillin/streptomycin and 0.5% L-glutamine (1% L-glutamine for MRC-5 cells). Cells were maintained in culture dishes at 37 °C in a 5% CO<sub>2</sub> and 95% air atmosphere incubator.

### Synthesis of organotin compounds 1–5

The Organotin(IV) complexes 1–5 were synthesised according to the method described earlier (Abdellah et al. 2009). Thus, a suspension of the ligand (2-hydroxybenzoic acid or 4-hydroxybenzoic acid) in distilled water was treated with an equimolar amount of KOH, and a clear solution was immediately formed. A methanolic solution of di-organotin(IV) or tri-organotin(IV) chloride was then added to the above solution. The white precipitation formed is filtered off, washed with water and it dried in vacuo over silica gel. The reaction route for the preparation is shown in Fig. 1. The analytical and spectroscopic data (m.p., elemental analysis, FT-IR, 1H-NMR and ESI-MS) of 1–5 are identical to those reported in reference Abdellah et al.

(2009) (Table 1). The molecular diagram of complexes 1 and 2 is presented in Fig. 2.

### Cell proliferation assay

Cell viability was evaluated using MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay. Cancer cells (LMS, HeLa, MCF-7 and U2-OS) and normal cells (MRC-5) were cultured at a density of  $3 \times 10^3$  and  $5 \times 10^3$ , respectively, in 96-well plates for 24 h. There were then washed with PBS and a solution containing different concentrations of complexes 1–5 or cisplatin (diluted in DMSO) was added. After incubation for another 48 h, 50 µl of MTT were added in each well from a stock solution (5 mg/ml) and the cells were incubated for another 4 h at 37 °C. The formed formazan crystals were solubilized with 50 µl of DMSO under gentle shaking and absorbance was determined at 570 nm (subtract background absorbance measured at 690 nm) using a microplate spectrophotometer (Multiskan Spectrum, Thermo Fisher Scientific, Waltham, USA). Data were expressed as a percentage of control measured in the absence of complexes (DMSO control). All experiments were performed in triplicate.

### Colony formation efficiency assay

LMS, HeLa, MCF-7 and U2-OS cells were seeded into six-well plates at a density of  $6 \times 10^4$  cells per well and incubated for 24 h. Cells were then washed with PBS, treated with media containing various concentrations of complexes 1–5 in DMSO and incubated for 48 h. LMS cells were also treated with cisplatin (0.5, 1 and 2.5 µM). Supernatants and cells were collected and counted using Neubauer cytometer and  $5 \times 10^2$  cells were seeded onto six-well plates. After 7 days, dishes were fixed with methanol–acetic acid 3:1 at –20 °C for 10 min, dried and stained with Giemsa dye 1:10 for 30 min. The dishes were then washed, dried and the colonies were counted.

### Quantification of apoptosis

LMS cells were seeded into six-well plates at a density of  $6 \times 10^4$  cells per well and incubated for 24 h before the experiment. Cells were washed with PBS, treated with media containing various concentrations of complexes 1–5 diluted in DMSO and cisplatin (IC<sub>50</sub> value) and incubated for 48 h. Supernatants and cells were collected, centrifuged and cell pellets were suspended in calcium buffer  $1 \times$  at a rate  $10^5$  cells/100 µl. Cells were stained with 5 µl of Annexin V-fluorescein isothiocyanate and 4 µl of propidium iodide in a dark room for 15 min. DNA content was determined on a FACScan flow cytometer (Partec ML, Partec GmbH, Germany).

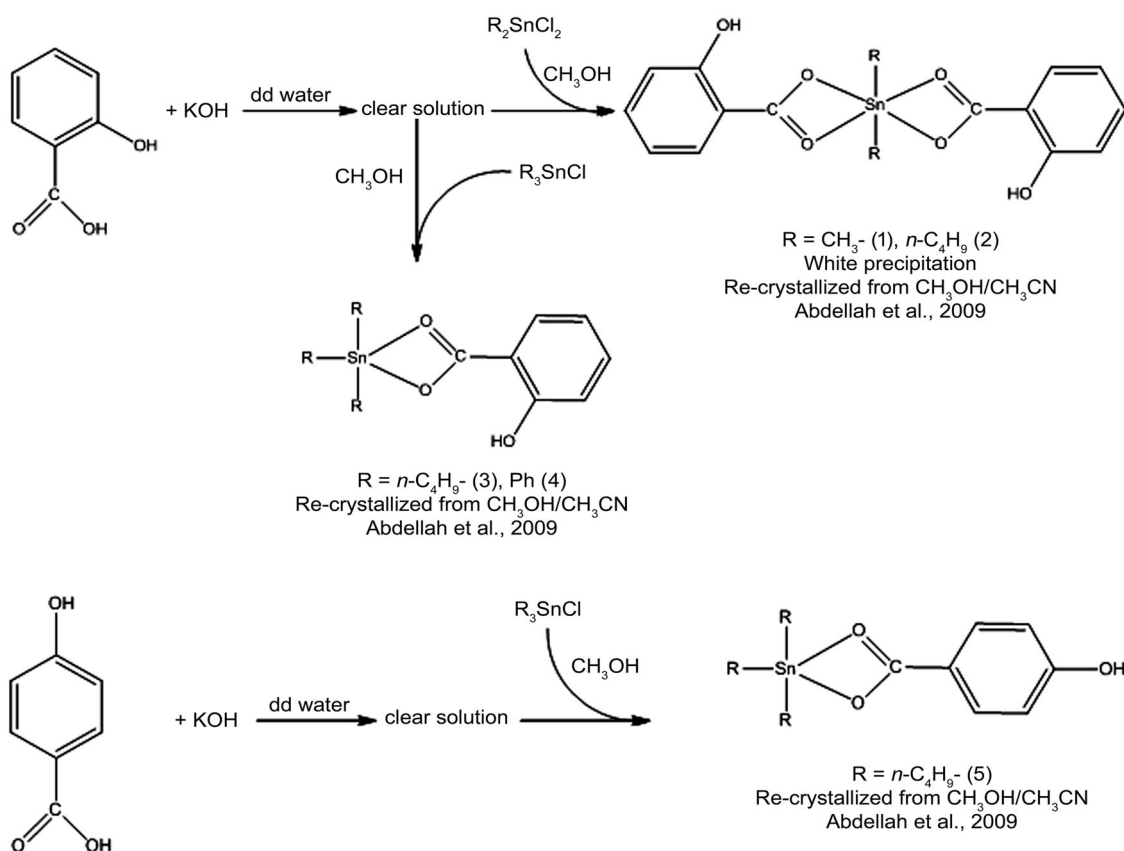
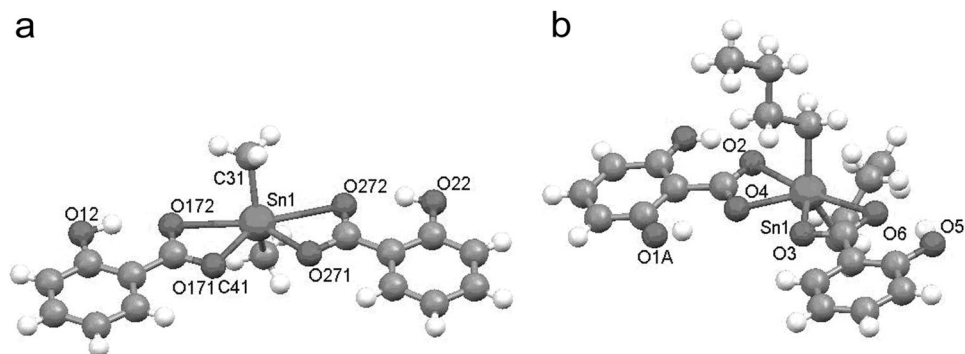


Fig. 1 Reaction route for the preparation of 1–5 (Abdellah et al. 2009)

Table 1 Characteristic vibration bands ( $\text{cm}^{-1}$ ) and  $^{119}\text{Sn}$  Mossbauer spectroscopic data for complexes 1–5 (Abdellah et al. 2009)

	Compound					
	1	2	3	4	5	
Infrared	$\nu$ (OH)	3467	3450	3450	3447	3439
	$\nu$ (CH)	2817–2705	2928–2869	2957–2364	3063	2958–2922
	$\nu_{\text{as}}, \nu_{\text{sy}}$ (COO)	1631, 1388	1628, 1419	1633, 1352	1636, 1356	1613, 1351
	$\nu$ (Sn–C)	580, 536	562, 530	564, 538	600, 533	606, 509
Raman	$\nu$ (Sn–C)	446	435	445	420	457
	$\nu$ (Sn–O)	–	–	–	–	410
	$\nu$ (Sn–C)	–	–	–	–	520
	$\delta$ ( $\text{mm s}^{-1}$ )	1.22	1.31	1.34	1.24	1.44
	$\Delta$ ( $\text{mm s}^{-1}$ )	3.09	3.53	2.93	2.48	3.18
	C–Sn–C ( $^\circ$ ) angles	130	143			
	Area (%)	56	29	100	100	100
	$\delta$ ( $\text{mm s}^{-1}$ )	1.33	1.56	–	–	–
	$\Delta$ ( $\text{mm s}^{-1}$ )	3.65	3.68	–	–	–
	C–Sn–C ( $^\circ$ ) angles	148	149			
Area (%)	44	71	–	–	–	

**Fig. 2** Molecular diagram with atomic numbering of **a** [Me<sub>2</sub>Sn(o-BZA)<sub>2</sub>] (**1**) and **b** [(n-Bu)<sub>2</sub>Sn(o-BZA)<sub>2</sub>] (**2**)



### DNA fragmentation assay

DNA fragmentation was estimated as described by Verginadis et al. (Verginadis et al. 2011). Briefly, LMS cells were treated with complexes **1–5** for 48 h. Supernatants and cells were collected and incubated with lysis buffer. Fragmented DNA was digested with RNase and Proteinase K and precipitated with isopropanol. Finally, DNA fragments were fractionated by 1.2% agarose gel and were visualised by ethidium bromide under ultraviolet light.

### Animals and treatment

Female Wistar rats, aged 3–4 months and weighting  $242 \pm 13$  g, were reared at controlled room temperature ( $20 \pm 2$  °C), with periodical lighting (12 h light/12 h dark). Standard Wistar rat diet and water ad libitum were used in all the experiments. Experiments on animals were handled with human care in accordance with the European Union directive for the care and the use of laboratory animals (EEC Directive 2010/63/EU) and according to the permission number 20EEP02.

### Acute toxicity

Forty female Wistar rats were divided into four groups (ten animals per group) and injected intra-peritoneally (i.p.) with a single dose of 1, 10 and 100 mg/kg body wt of complex **1** in order to achieve a logarithmic increase of the dose. Control group was injected with a similar volume of the solvent (1 ml of PBS/DMSO (7:1)). All animals were starved for 3–4 h before treatment. Twenty minutes after the administration, the rats were allowed to access freely food and water. The animals were observed for their behaviour twice a day. Body weight (body wt) was recorded every 2 days and used as an index of toxicity. The percentage change in body wt was calculated as:  $\text{Body wt (\%)} = \frac{[\text{mean body wt on day } D - \text{mean body wt on day } 1]}{\text{mean body wt on day } 1} \times 100$ , where  $D = \text{day of the experiment}$ .

The animals survived were sacrificed by ether on day 15. The internal organs were removed en bloc and fixed in 10% buffered formalin. Tissue sections of formalin-fixed, paraffin-embedded tumours were stained with haematoxylin–eosin. The sections were examined under light microscope.

### Subacute toxicity

Subacute toxicity was studied in 30 female Wistar rats that were divided into three groups (10 animals per group), and received i.p. daily dosages of solvent (0.5 ml), 0.5 and 1 mg/kg body wt of complex **1** for 4 weeks. Animals were monitored for their behaviour once per day. Body weight was recorded every 2 days and was used as an index of toxicity, while the percentage change in body wt was calculated as previously. All animals were sacrificed by ether at the end of experiment. Immediate autopsy, fixation of the organs in 10% buffered formalin and a histopathological examination were performed as described previously (Verginadis et al. 2011).

### Antitumour activity in vivo

Twenty-nine female Wistar rats were inoculated with  $3 \times 10^6$  LMS cells/1 ml Hank's balanced salt solution on the upper scapula after anaesthetisation (mixture of anaesthesia was composed of ketamine 10% (Imalgene), midazolame 40% (Roche) and saline solution 0.9, 50% to a total volume of 0.5 ml per animal). After the appearance of a palpable tumour mass (smaller than 1.5 cm diameter), the inoculated animals were divided into two groups: the control group (CG, consisted of 15 animals) and the treatment group (TG, consisted of 14 animals). All animals in TG Group were treated daily till death with 1 mg/kg body wt of complex **1** (diluted in 0.5 ml PBS/DMSO 7:1), while the animals of the CG received daily 0.5 ml PBS/DMSO(7:1). The animals were observed for their behaviour once a day. After the animals' death, an autopsy, fixation of the organs in 10% formaldehyde and a histological examination were

performed. For both groups, the MST of the animals, tumour weight inhibition (TWI%), mean tumour weight and mean tumour growth rate (MTGR) were calculated. The tumour growth rate for each animal was calculated by the following equation: Tumour growth rate (g/d) = tumour weight (g)/survival time (days), while TWI% was calculated as:  $TWI\% = [(mean\ control\ tumour\ weight - mean\ treatment\ tumour\ weight) / mean\ control\ tumour\ weight] \times 100$ .

### Statistical analysis

The data are expressed as mean  $\pm$  S.D., and the difference between two groups was evaluated using Student's *t*-test and two-way analysis of variance (SPSS version 17.0, Statistical Package for the social Sciences software, SPSS, Chicago, USA). A probability level of  $p < 0.05$  was used to establish significance.

## Results

### Cytotoxicity

Complexes 1–5 were tested for their in vitro cytotoxic activity against all cell lines with the MTT assay. The IC<sub>50</sub> values for cell growth proliferation after 48 h of incubation were calculated. Cisplatin was used as the reference treatment. Moreover, the survival of MRC-5 cells, at the IC<sub>50</sub> values of the complexes against the LMS cells, was also calculated.

All complexes showed potent cytotoxic activity against the three human cells lines ranging from 108 to 724 nM for the MCF-7 cells, 21 to 295 nM for the HeLa cells and 97 to 820 nM for the U2-OS cells. The IC<sub>50</sub> value of complex 2 was 304 and 211 times lower than the corresponding value of cisplatin for the MCF-7 and U2-OS cells, respectively while the IC<sub>50</sub> value of complex 4 was 264 times lower for the HeLa cells. Among the tested compounds, complexes 3 and 5 presented the weaker cytotoxic activity (Table 2).

**Table 2** IC<sub>50</sub> values for cell viability calculated for complexes 1–5 and cisplatin against MCF-7 (breast cancer), HeLa (cervical cancer) and U2-OS cells (osteosarcoma)

Compound	IC <sub>50</sub> (nM)		
	MCF-7	HeLa	U2-OS
1	142 $\pm$ 4.3	84 $\pm$ 3.2	130 $\pm$ 2.3
2	108 $\pm$ 2.6	37 $\pm$ 1.2	97 $\pm$ 2.1
3	724 $\pm$ 5.4	295 $\pm$ 3.8	820 $\pm$ 5.7
4	121 $\pm$ 3.7	21 $\pm$ 1.1	258 $\pm$ 3.2
5	325 $\pm$ 2.3	120 $\pm$ 2.9	535 $\pm$ 4.6
Cisplatin	32850 $\pm$ 2390	5540 $\pm$ 680	20510 $\pm$ 1250

Furthermore, the cytotoxic activities of complexes 1–5 and cisplatin was studied in LMS cells. Complex 1 presented the lower IC<sub>50</sub> (31 nM) followed by complex 2 (43 nM) and 4 (45 nM). The IC<sub>50</sub> values for complexes 3 and 5 were higher than the other 3 complexes but still extremely lower compared to cisplatin. Trypan blue assay was previously performed by Abdellah et al. (2009) in order to determine the cell membrane integrity. The complexes have high activity against LMS cells while complex 4 was the most active one. Except from complex 5 the rest of the complexes exhibited no inhibitory activity on growth proliferation of MRC-5 cells (IC<sub>50</sub> concentration of complexes against LMS cells) (Table 3).

### Colony formation efficiency after treatment

The cells were tested for their ability to form colonies after drug treatment compared to untreated cells. Complexes 1 and 4 presented a dose-dependent inhibition of MCF-7, HeLa and U2-OS cells colony formation. All human cancer cell lines treated with dosages higher than 20 nM of complex 2 lost their ability to form colonies (Fig. 3)

After treatment with complexes 1 and 2, LMS cells gradually lost their ability to form colonies. Complex 2 at the highest dose (100 nM) completely abolished the formation of colonies while LMS cells resisted treatment with complex 4 and were able to form colonies even at the highest concentration of the complex (Fig. 4a). Complexes 3 and 5 failed to inhibit colony formation at concentrations lower than 100 nM (results not shown). On the other hand, cisplatin also exerted a dose-dependent inhibition of colony formation of LMS cells but at significantly higher doses than complexes 1, 2 and 4 (Fig. 4b).

### Flow cytometry and DNA fragmentation

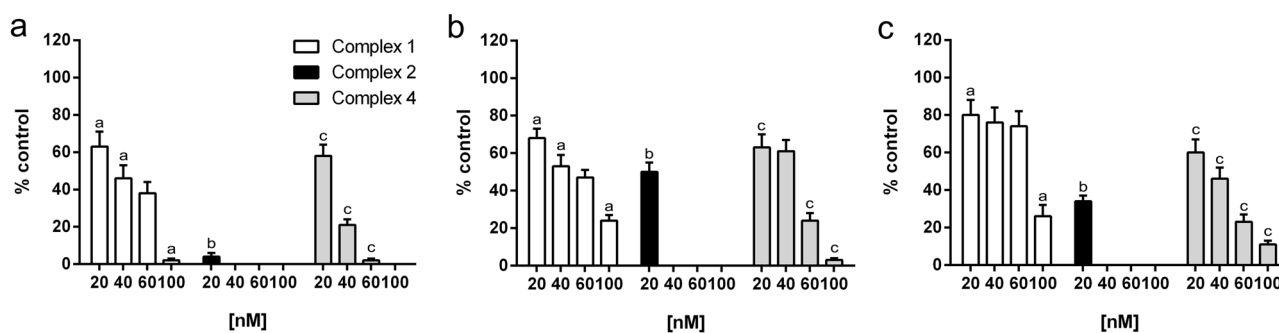
Flow cytometry assay was used to quantify apoptotic or necrotic death in LMS cells treated with compounds 1–5 or

**Table 3** Cytotoxic activity of complexes 1–5 and cisplatin against LMS cells after 48-h incubation (IC<sub>50</sub> values) and survival of MRC-5 cells

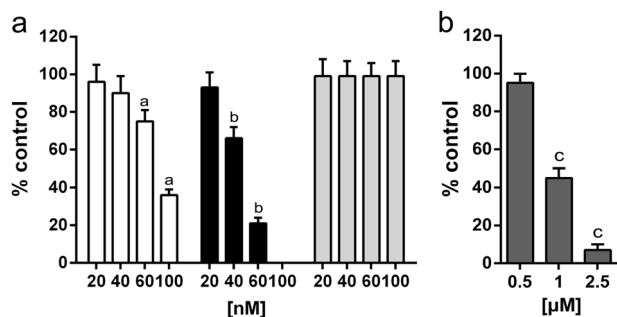
Complex	LMS		MRC-5
	IC <sub>50</sub> (nM)	MTT	% Survival (LMS IC <sub>50</sub> value)
1	31 $\pm$ 1.6	>2000	97.5 $\pm$ 0.15
2	43 $\pm$ 1.5	150	104.1 $\pm$ 0.2
3	738 $\pm$ 2.4	150	98.1 $\pm$ 0.1
4	45 $\pm$ 1.8	5–10	94.5 $\pm$ 0.2
5	134 $\pm$ 2.8	30–40	78.4 $\pm$ 0.2
Cisplatin	5480 $\pm$ 530	5000	

<sup>a</sup>Abdellah et al. (2009)





**Fig. 3** Formation of colonies for **a** MCF-7, **b** HeLa and **c** U2-OS cells after treatment with the complexes **1**, **2** and **4**. Different letters indicate the significant differences ( $p < 0.05$ ) among the current and the exact previous (lower) dose



**Fig. 4** Formation of colonies for LMS cells after treatment with **a** the complexes **1**, **2** and **4** and **b** cisplatin. Different letters indicate the significant differences ( $p < 0.05$ ) among the current and the exact previous (lower) dose

the  $IC_{50}$  of cisplatin ( $5.5 \mu\text{M}$ ). Compared to untreated LMS cells that showed a total of  $6.2 \pm 1.3$  and  $1.7 \pm 0.5\%$  apoptotic and necrotic cell death, respectively, the cells treated with complex **1** at  $50 \text{ nM}$  showed  $22.4 \pm 1.8\%$  apoptosis and  $2.1 \pm 0.2\%$  necrosis. At higher concentrations ( $100 \text{ nM}$ ), the cells showed  $21.1 \pm 2.8\%$  apoptosis and  $1.6 \pm 0.6\%$  necrosis. As for complexes **2** and **4**, cells showed  $33.8 \pm 2.3$  and  $13.7 \pm 2.6$  apoptosis and  $1.8 \pm 0.1$  and  $2.2 \pm 1.4\%$  necrosis after treatment with  $50 \text{ nM}$  of the complexes, respectively and  $38.7 \pm 2.2$  and  $28.9 \pm 1.6\%$  apoptosis and  $7.5 \pm 0.9$  and  $2.8 \pm 1.2\%$  necrosis after treatment with  $100 \text{ nM}$ . Complexes **3** and **5** induced apoptosis of LMS cells at higher concentrations ( $>300 \text{ nM}$ ) (results not shown). Apoptosis induced by cisplatin ( $IC_{50}$  value) was estimated at  $35.0 \pm 3.0\%$  and necrosis at  $3.3 \pm 1.6\%$  (Fig. 5a, b).

The characteristic laddering pattern observed at the electrophoresis gel after treatment of LMS cells with  $150$  and  $100 \text{ nM}$  of complexes **1** and **2** and **4**, respectively, indicated that cells underwent apoptosis triggered by the DNA damage and fragmentation (Fig. 5c).

**Acute toxicity**

Ninety percent of the animals at the highest dosage group ( $100 \text{ mg/kg}$  body wt) died within an hour after the

i.p. administration of complex **1**, while  $40\%$  of the animals that received  $10 \text{ mg/kg}$  body wt died within  $24 \text{ h}$ . None of the animals from the other two groups (control and  $1 \text{ mg/kg}$  body wt) died during the experiment (Table 4).

All animals that received  $1 \text{ mg/kg}$  body wt of complex **1** did not show any signs of toxicity. The absence of toxicity was verified by the histologic examination. Animals injected with  $10 \text{ mg/kg}$  body wt of complex **1** showed reduced mobility and difficulty in breathing during the first  $24 \text{ h}$ . Animals presented intent seizures right after the administration of the complex **1** at the highest dose ( $100 \text{ mg/kg}$  body wt). The main organs targeted were liver, kidneys and lungs. The diseased animals showed cerebral oedema as well (Fig. 6).

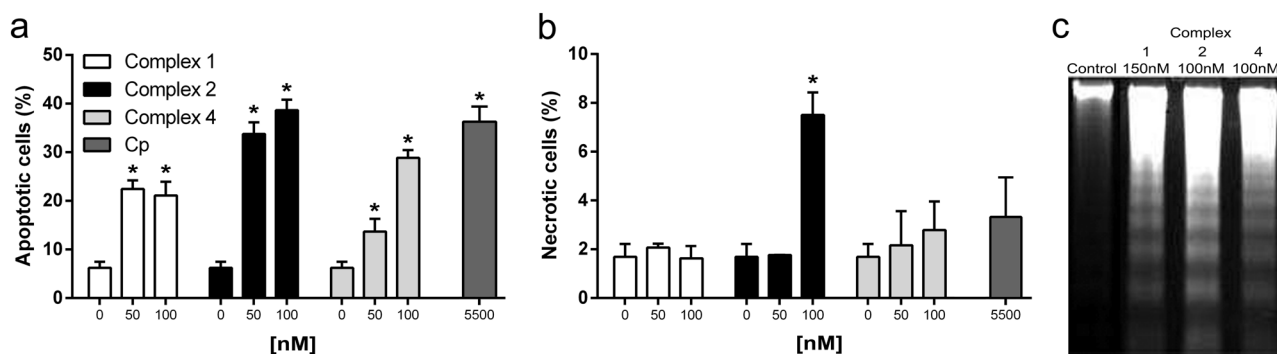
**Subacute toxicity**

None of the animals presented any signs of toxicity. Mild kidney damage was only observed at animals treated daily with the highest dose ( $1 \text{ mg/kg}$  body wt of complex **1**) (results not shown).

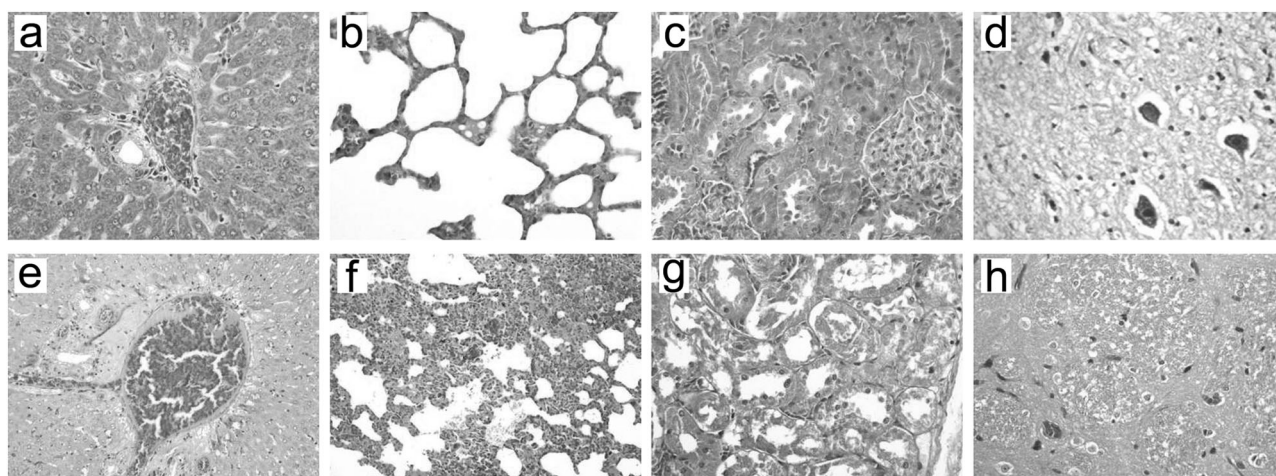
**Antitumour activity**

Daily administration of tumour bearing Wistar rats with  $1 \text{ mg/kg}$  with complex **1** until death prolonged the MST ( $120\%$  increase) and reduced the MTGR by more than  $3\times$  fold and with minimal side effects (Table 5). Furthermore tumour weight inhibition was calculated as  $TWI\% = 60.2\%$ . Long terms survival was noted for  $LTS = 1/15$ . This animal belonged in TG group and was excluded from the above calculations.

It is worth mentioning that total tumour regression was observed in one animal treated with complex **1**. The histopathological examination showed an abscess cavity at the site of the inoculation. This animal was the only one with an observed lung metastasis, which was resistant to treatment.



**Fig. 5** Quantification of apoptotic and necrotic cells after treatment with 50 and 100 nM of complex **1**, **2** and **4** and cisplatin (CP, 5.5  $\mu$ M) for 48 h. \*Statistically significant from untreated cells,  $p < 0.05$ . Data are presented as mean  $\pm$  SD



**Fig. 6** Acute toxicity report. Healthy samples from the control group: **a** liver, **b** lung, **c** kidney and **d** brain stem and representative histological images from animals that received 100 mg/kg body wt of complex **1**: **e**

liver, **f** lung, **g** kidney and **h** brain stem. Tissue sections were stained with Hematoxylin–eosin. Liver and lung sections were magnified by  $\times 200$  while kidney and brain stem sections by  $\times 400$

**Table 4** Toxic effects (acute toxicity) induced by complex **1**

Group	Kidney	Liver	Lung	Brain stem	$\Delta$ Body wt (%)	Death (%)
Control	(–)	(–)	(–)	(–)	$+2.01 \pm 0.10$	0
1 mg/kg body wt	(–)	(–)	(–)	(–)	$+1.37 \pm 0.32$	0
10 mg/kg body wt	Oedema	Oedema	(+)	(–)	$-2.40 \pm 0.23$	40
100 mg/kg body wt	(+)	(+)	(+)	(+)	$-0.10 \pm 0.00$	90

Body wt (%) = [(mean body wt on day  $D$  – mean body wt on day 1)/mean body wt on day 1]  $\times$  100, where  $D$  = day of the experiment  
(–) absent, (+) mild histological alterations

## Discussion

Ever since cisplatin was introduced in cancer therapy, many non-conventional platinum compounds and non-platinum metal complexes were designed and tested. Among them, organotin derivatives have shown potent cytotoxic and antitumour properties (Arjmand et al. 2014).

Verginadis et al. showed that a triorganotin complex with 2-mercapto-nicotinic acid inhibits LMS cell proliferation

with the corresponding  $IC_{50}$  value of the complex being significantly lower than that of cisplatin (41 nM versus 25  $\mu$ M) (Verginadis et al. 2011). Di-*n*-butyltin(IV) compounds related to salicylic acid were found to be 4–10 $\times$ -fold more effective than cisplatin against A204, MCF-7 and T24 human tumour cells (Ross et al. 1991). This is in accordance with our results where all complexes exerted higher cytotoxic activity than cisplatin against MCF-7, HeLa, U2-OS and LMS cells.

**Table 5** Mean survival time and mean tumour growth rate of tumour bearing Wistar rats

	Control group ( <i>N</i> = 15)	Treatment group ( <i>N</i> = 14)
MST (days)	20.7 ± 5.5	45.1 ± 5.9*
Mean tumour weight (g)	108 ± 25	72 ± 9*
MTGR (g/day)	5.2 ± 0.2	1.6 ± 0.1*

MST mean survival time, MTGR mean tumour growth rate

\*Statistically significant from the control group,  $p < 0.05$ . Data are presented as mean ± SD

Flow cytometry revealed that complexes **1**, **2** and **4** caused LMS cell death through apoptosis and inhibited human cancer cells and LMS cell's ability to form colonies. Moreover, electrophoresis of the LMS cell's DNA showed that apoptosis was triggered by the DNA damage and fragmentation after incubation with complexes **1**, **2** and **4**. Such DNA damage is shown for several organotin derivatives for instance tributyltin (Liu et al. 2006). The inability of complexes **3** and **5** to induce apoptosis at similar, low doses as complexes **1**, **2**, and **4** as well as to inhibit colony formation could be possibly attributed to an alternative apoptotic mechanism and specifically to the rise of intracellular  $\text{Ca}^{2+}$  that would lead the cell to apoptosis (Nakatsu et al. 2007; Reader et al. 1999; Viviani et al. 1995) but would also allow the cells to develop after the complex was removed from the culture media. This is only a hypothesis that would need additional experiments to be verified although the relative lower cytotoxic activity of complexes **3** and **5** compared to **1**, **2**, and **4** suggest that they should be excluded from further research. Apoptosis seems to be the main type of cell death caused by many organotin derivatives even though the path that leads to apoptosis may differ between different complexes (Nakatsu et al. 2007; Grondin et al. 2007; Tada-Oikawa et al. 2008). Diorganotin(IV) and triorganotin(IV) complexes of meso-tetra(4-sulfonatophenyl) porphine induce apoptosis in A375 human melanoma cells via both death receptor-mediated and mitochondrial apoptotic pathways (Amir et al. 2014), while triphenyl tin benzimidazolethiol induces mitochondrial-mediated apoptosis in human cervical cancer cells (Hoti et al. 2003). On the other hand, trimethyl tin seems to cause both apoptotic and necrotic death in human hepatoma G2 cells (Cai et al. 2009).

Organotin derivatives are known to cause neurotoxicity and bile duct damage (Boyer 1989; Winship 1988). Complex **1** caused the death of 90% of the animals after a single dose of 100 mg/kg within an hour of the i.p. administration. Since the main pathological findings (brain stem and lung oedema and mild liver and kidney damage) were not capable to cause the animal's death, a possible cause was

metabolic acidosis (Matsuno-Yagi and Hatefi 1993). This is a very likely explanation considering that all animals presented Kussmaul type of breath before death. Similar findings were reported after i.p. administration of triphenyltin 2-(triphenyltinmercapto) nicotinate with kidney damage being more severe reaching total tubular necrosis (Verginadis et al. 2011). A simple i.p. administration of the complex at lower doses (10 mg/kg BW) showed toxic effects and caused death to the 40% of the animals within 24 h. Repeated administration of the complex at very low doses (1 mg/kg/day) for 30 days caused only slight kidney damage. The same dosage was used to study the antitumour effects of the complex.

Tumour-bearing animals treated with complex **1** showed prolongation of survival time and tumour growth inhibition. Other organotin derivatives have higher TWI% values (Verginadis et al. 2011), while oral administration of triethyltin(IV)-lupinylsulfide hydrochloride showed as high as 90% tumour volume inhibition (Muhammad and Guo 2014). Not all organotin complexes are active in vivo. Organotin complexes with salicylic acid derivatives are inactive against L1210 leukaemia in mice (Gielen et al. 1993), while triphenyltin 5-sulfosalicylate and triphenyltin 5-aminosalicylate offer no protection against murine colon carcinoma colon 26 (Gielen 1995). Both treated and untreated animals showed slight liver damage and minor lung oedema, which could be attributed to the tumour, but only treated animals showed kidney damage, as expected by the subacute toxicity results. It is noteworthy to mention that one of the treated animals showed total tumour regression at the site of the inoculation. Unfortunately, this animal was the only one presenting lung metastasis. The lung metastasis presented to this animal was resistant to treatment and showed no signs of necrosis during the histological evaluation.

## Conclusions

In this study, we evaluated the cytotoxic and antitumour properties of five organotin complexes (**1**–**5**) with *o*-hydroxy-benzoic or *p*-hydroxy-benzoic acids. Amongst them, complex **1** presented the highest cytotoxic activity against LMS cells ( $\text{IC}_{50} = 31 \pm 1.6$  nM), complex **2** against MCF-7 and U2-OS cells ( $\text{IC}_{50} = 108 \pm 2.6$  nM and  $97 \pm 2.1$  nM, respectively) and complex **4** against HeLa cells ( $\text{IC}_{50} = 21 \pm 1.1$  nM). The induction of apoptosis was identified as the mechanism of LMS cell death. Complexes **1** and **2** showed a higher inhibitory effect on LMS colony formation efficiency compared to complex **4**. The following acute toxicity study revealed a mildly toxic effect of complex **1** at a dose of 10 mg/kg body weight in the liver, kidney and lungs of adult Wistar rats. Tumour-bearing



Wistar rats administrated daily with 1 mg/kg body weight of complex **1** lived longer than the control rats (45.1 versus 20.7 days) probably due to the deceleration of tumour growth rate (1.6 versus 5.2 g/day) caused by the complex. In short, our results demonstrated that complex **1** exerted potent cytotoxic activity, tumour growth retardation and prolongation of MST in tumour-bearing (LMS) Wistar rats. Thus, it could be used as a potential candidate for cancer treatment at primary site of the tumour. Further investigation is needed to confirm the antitumour properties of the other organotin complexes.

### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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