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Research paper

A novel compound of triphenyltin(IV) with *N-tert*-butoxycarbonyl-L-ornithine causes cancer cell death by inducing a p53-dependent activation of the mitochondrial pathway of apoptosis

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ABSTRACT

The triphenyltin(IV) compound with *N-tert*-butoxycarbonyl-L-ornithine (Boc-Orn-OH), [Ph_3Sn(Boc-Orn-O)], was synthesized and characterized by elemental analysis, FT-IR, solution ^{1}H, ^{13}C and ^{119}Sn NMR and ESI mass spectrometry. The organotin(IV) compound inhibited at very low micromolar concentrations the growth of human tumor cell lines HepG2 (hepatocarcinoma cells), MCF-7 (mammary cancer) and HCT116 (colorectal carcinoma) while it did not affect the viability of non-malignant human-derived hepatic cells Chang. The mechanism of the antiproliferative effect of Ph_3Sn(Boc-Orn-O), investigated on human hepatoma HepG2 cells, was pro-apoptotic, being associated with externalization of plasma membrane phosphatidylserine, chromatin condensation or fragmentation and mitochondrial dysfunction as well as with increase of p53 levels.

1. Introduction

Organotin(IV) derivatives have been prepared and tested in the past as possible anticancer agents [1–6] and many of them exhibited interesting activity in specific cancer models. Tin-based drugs represent an excellent alternative to platinum ones as antitumor agents, having the considerable advantage to display a lower toxicity. Triorganotins have demonstrated potential antiproliferative activity *in vitro* [7] against human tumor cell lines, which has been related to their ability to bind to proteins [8,9]. Special attention is given to organotin(IV) carboxylates with significant cytotoxic properties against different cancer cell lines [10,11]. Some compounds have shown strong apoptosis inducing character *in vitro*, which can be higher than the corresponding activity of cisplatin or other clinical anticancer drugs [12].

Although the majority of organotin(IV) compounds cause apoptotic cell death, the exact mechanism of action is not yet clearly determined [13]. Several researches have been focused on understanding the binding mode of organotin(IV) compounds to biologically relevant ligands [3,14], often using small peptides, as low-molecular-weight proteins to mimic the metal ion interaction. The organic ligand facilitates the transport of the compounds across the cell membrane, while the antitumor activity is due to dissociated organotin(IV) moieties.

On account of their structural variability, organotin(IV) derivatives of *N*-substituted amino acids have been extensively studied [14]. Moreover, also organotin(IV) carboxylates of *N*-protected amino acids have shown interesting pharmacological applications as antitumor agents. Thus, it is of particular interest to examine the structural variations caused by protecting groups on the amino nitrogen of the ligand [15]. One of the most widely used *N*-terminal protecting groups is the *tert*-butoxycarbonyl (Boc) group. Boc-amino acids are often used as substrates, substrate analogues or competitive inhibitors of proteolytic enzymes. The study of their conformational preferences is also important for understanding their interactions with enzymes [16].

Several reports have been published concerning structural characterization and bioactivity as antitumor agents of di- and tri-organotin(IV) compounds [17]. Following our previous

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investigations on organotin(IV) compounds of L-arginine and *N-tert*-butoxycarbonyl-L-arginine [18,19], we extended our work to the synthesis, structural characterization and biological activity of a triphenyltin(IV) derivative with *N-tert*-butoxycarbonyl-L-ornithine (Boc-Orn-OH) (Fig. 1).

Ph_3Sn(Boc-Orn-O) has been synthesized and characterized by elemental analysis, FT-IR, ESI mass spectrometry, ^1H, ^{13}C and ^{119}Sn NMR spectroscopic techniques and its cytotoxic behavior has been investigated on three human tumor cell lines, HepG2 (hepatocarcinoma cells), MCF-7 (mammary cancer) and HCT116 (colorectal carcinoma) as well as on non-malignant human-derived hepatic cells (Chang). Moreover, the mechanism of its anti-tumor activity has been evaluated.

2. Experimental

2.1. Materials and physical measurements

Ph_3SnOH (Aldrich) and *N-tert*-butoxycarbonyl-L-ornithine (Fluka) were used without further purification. Elemental micro-analyses for C, H and N were performed at the Laboratorio di Microanalisi, University of Padova, Italy. Infrared spectra were recorded with a Perkin-Elmer Spectrum One FT-IR spectrometer, using KBr disc with a resolution of 4 cm^{-1}. All NMR spectra were acquired with an Avance II DMX 400 MHz (9.40 T) spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany), operating at 400.15 MHz for protons, 100.63 MHz for ^{13}C, and 149.20 MHz for ^{119}Sn. One-dimensional ^1H and $^{13}C\{^1H\}$ spectra in CD_3OD and DMSO-d_6 solutions were acquired at 27 °C with a spectral width (*i.e.* SW) of 12 ppm and 200 ppm, respectively. One-dimensional $^{119}Sn\{^1H\}$ NMR spectra in CD_3OD and DMSO-d_6 solutions were recorded at 27 °C with a SW of 800 ppm by investigating four spectral windows with SW = 250 ppm at once in the +200 to −600 ppm range. For ^{119}Sn, Me_4Sn was employed as external reference (^{119}Sn, δ = 0.00 ppm). ^1H and ^{13}C resonances were calibrated on Me_4Si as external reference (^1H, δ = 0.00 ppm; ^{13}C, δ = 0.00 ppm). $^{119}Sn\{^1H\}$ and $^{13}C\{^1H\}$ spectra were acquired with broadband proton power-gated decoupling. For all nuclei, positive chemical shift had higher frequencies than the reference. LW in the text is intended as line width at half height. Solutions concentrations were ca. $0.5\cdot10^{-3}$ M. Solid-state $^{119}Sn\{^1H\}$ CP-MAS spectra were acquired with NS (*i.e.* number of transients) equal to 1200, using recycling delays of 2 s, contact time of 6 ms and acquisiton time of 11.5 ms. Spinning rates of 5 kHz and 7 kHz were used in order to get the isotropic $\delta^{119}Sn\{^1H\}$value. Line broadening of 75 Hz and zero filling of 16 k points were applied to the free induction decays (FID) before transformation. After calibration on the $(c\text{-}C_6H_{12})_4Sn$, a power level of 4 dB was applied in order to get the Hartman–Hahn condition. 119Sn chemical shifts are given with respect to the solid $(c\text{-}C_6H_{12})_4Sn$ as secondary reference [δ (^{119}Sn) = −97.0 ppm with respect to Me_4Sn]. Solid-state $^{13}C\{^1H\}$ CP-MAS spectra were acquired with NS = 512 using recycling delays of 3 s, contact time of 2 ms and acquisiton time of 34 ms. Spinning rates of 5 kHz and 8 kHz were used in order to get the isotropic $\delta(^{13}C)$ value. Line broadening of 25 Hz and zero filling of 4 k points were applied to the FID before transformation. A power level of 5.1 dB was applied in order to get the Hartman–Hahn condition. ^{13}C chemical shifts are given with respect to the solid admantane as secondary reference [δ ($^{13}CH_2$) = 29.5 ppm; $\delta(^{13}CH)$ = 38.6 ppm with respect to Me_4Si]. Positive-ion and negative-ion electrospray ionization (ESI) mass spectra were measured on an ion trap analyzer Bruker 3000 +in the range *m*/*z* 60–1500. The compound was dissolved in methanol or acetonitrile, diluted at 0.1–1.0 mg/mL in MeOH/H_2O and acetonitrile/H_2O (80/20), and analyzed in ESI-MS by direct infusion at a flow rate of 6 µl/min.

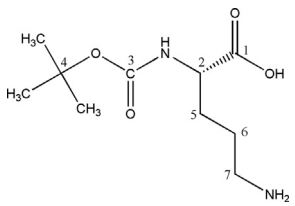

Fig. 1. *N-tert*-butoxycarbonyl-L-ornithine (Boc-Orn-OH).

2.1.1. Synthesis of Ph_3Sn(Boc-Orn-O)

The reaction of Ph_3SnOH with Boc-Orn-OH in a 1:1 molar ratio, led to the formation of the organotin(IV) compound according to Eq. (1)

$$Ph_3SnOH + \text{Boc-Orn-OH} \rightarrow Ph_3Sn(\text{Boc-Orn-O}) + H_2O \quad (1)$$

A solution of Ph_3SnOH (0.734 g, 2 mmol) in dry methanol (15 mL) was added drop wise to a methanol solution (15 mL) of the ligand (Boc-Orn-OH, 0.464 g, 2 mmol) and left to react, under stirring, for 4 h. After cooling at room temperature, the solvent was reduced under *vacuum* to a small volume (5 mL) using a rotary evaporator; a white solid residual was obtained, which was filtered off, washed three times with a total amount of methanol of 50 mL and dried *in vacuo* in presence of P_4O_{10}. $C_{28}H_{34}N_2O_4Sn$: *M* = 581.31 g/mol; m.p. = 118 °C; Anal.Calc.: C 57.85, H 5.89, N 4.82, Sn 20.42%. Found: C 57.50, H 6.16, N 5.11, Sn 20.31%. IR (KBr, cm^{-1}): 3379 m, 3327w, 3263 m ν(NH); 1654 s $\nu_{as}(COO^-)$, 1413 m $\nu_s(COO^-)$, $\Delta\nu$ = 241; 280 m ν_{as}(sn-C), 227w ν_s(sn-C). ^1H NMR (400.15 MHz, CD_3OD): δ (ppm) 7.78–7.76 (br, 6H, *o*-protons in $SnPh_3$, $|^3J(^{119}Sn,^1H)|$ = 59.0 Hz); 7.49–7.42 (br, m, 9H, *m*- and *p*-protons in $SnPh_3$); 3.93 (br,1H, H-2); 2.88 (t, 2H, H-7); 1.81(m, 2H, H-5); 1.66 (m, 2H, H-6); 1.41 (s, 9H, Boc). ^{13}C NMR (100.63 MHz, CD_3OD): δ (ppm) 173.4 (C-1); 157.6–158.2 (C-3); 140.8–140.9 (Sn–C_6H_5 *ipso*, $^1J(^{119}Sn,^{13}C)|$ = 738 Hz); 137.7 (Sn–C_6H_5 *ortho*, $|^2J(^{119}Sn,C)|$ = 43.2 Hz); 129.7 (Sn–C_6H_5 *meta*, $|^3J(^{119}Sn,C)|$ = 64.4 Hz); 130.5–130.6 (Sn–C_6H_5 *para*, $|^4J(^{119}Sn,C)|$ = 13.4 Hz); 80.3 (C_{quat}-4); 52.5 (C-2); 42.7 (C-7); 29.3 (C-5); 28.8 (C-Boc); 22.3 (C-6). ^{119}Sn NMR (149.20 MHz, CD_3OD): δ (ppm) −166.8. ^1H NMR (400.15 MHz, DMSO-d_6): δ (ppm) 7.79–7.78 (d, 6H, *o*-protons in $SnPh_3$, $|^3J(^{119}Sn,C)|$ = 59.5 Hz); 7.40–7.38 (d, 9H, *m*- and *p*-protons in $SnPh_3$); 3.70 (d, 1H, H-2); 2.39 (t, 2H, H-7); 1.92–1.74 (m, 2H, H-5), 1.51 (d, 2H, H-6), 1.38 (s, 9H, Boc). ^{13}C NMR (100.63 MHz, DMSO-d_6): δ (ppm) 170.1 (C-1); 155.3 (C-3); 142.5–141.9 (Sn–C_6H_5 *ipso*); 136.1–135.9 (Sn–C_6H_5 *ortho*), $^2J(^{119}Sn,C)|$ = 42.4 Hz; 128.2 (Sn–C_6H_5 *meta*, $|^3J(^{119}Sn,C)|$ = 59.5 Hz; 128.9 (Sn–C_6H_5 *para*); 77.6 (C_{quat}-4); 50.2 (C-2); 40.9 (C-7); 28.2 (C-Boc); 27.8 (C-5); 21.1 (C-6). ^{119}Sn NMR (149.20 MHz, DMSO-d_6): δ (ppm) −125.9, −137.5. CP-MAS ^{13}C NMR (100.63 MHz) for the ligand: δ (ppm) 179.1 (C-1); 156.5 (C-3); 77.6 (C_{quat}-4); 58.5 (C-2); 40.7 (C-7); 30.9 (C-Boc); 29.2 (C-5); 25.4 (C-6); CP-MAS ^{13}C NMR (100.63 MHz) for the compound: δ (ppm) 179.5, 176.1, 171.8 (C-1); 157.4 (C-3); 146.8 (Sn–C_6H_5 *ipso*); 143.0 (Sn–C_6H_5 *ortho*); 128.6 (Sn–C_6H_5 *meta*); 137.8, 134.5 (Sn–C_6H_5 *para*); 77.2 (C_{quat}-4); 58.4, 52.9, 50.4 (C-2); 42.3, 40.7, 38.5 (C-7); 29.6 (C-Boc); 24.5 (C-5); 22.0 (C-6); CP-MAS ^{119}Sn NMR (149.20 MHz): δ (ppm) -297. ESI-MS: M_W = 582. Positive-ion MS: *m*/*z* 604.9 [M + Na]$^+$; *m*/*z* 582.9 [M + H]$^+$; Negative-ion MS: *m*/*z* 626.8 [M + HCOO]$^-$; *m*/*z* 580.9 [M-H]$^-$ traces.

Due to the low solubility of the ligand, $^{13}C\{^1H\}$ NMR spectra of Boc-Orn-OH and Ph_3Sn(Boc-Orn-O), were recorded also in solid state to check the compound formation.