Polyhedron 29 (2010) 613–619

Contents lists available at ScienceDirect

Polyhedron

journal homepage: www.elsevier.com/locate/poly

In vitro biological studies and structural elucidation of organotin(IV) derivatives of 6-nitropiperonylic acid: Crystal structure of $\{[(CH_2O_2C_6H_2(o\text{-}NO_2)COO)SnBu_2]_2O\}_2$

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ARTICLE INFO

Article history:
Available online 26 July 2009

Keywords:
Organotin(IV) compounds
6-Nitropiperonylic acid
Multinuclear NMR
Crystal structure
Distannoxane
Bioassay

ABSTRACT

Six organotin(IV) compounds with general formulae R_3SnL, R = Me (**2**), Ph (**6**), R_2SnL_2, R = Me (**1**), Et (**3**), *n*-Oct (**5**), $[((R_2SnL)_2O)_2]$, R = Bu (**4**), L = 6-nitropiperonylate, have been prepared. The newly synthesized compounds have been characterized by elemental analysis, FT-IR and multinuclear NMR (^1H, ^{13}C and ^{119} Sn). The structure of **4** has been determined by single crystal X-ray crystallography which is triclinic with the space group $P\bar{1}$. The crystal structure contains centrosymmetric dimer distannoxane with planar central four-membered Sn_2O_2 core. The 6-nitropiperonylate ligand shows different modes of coordination with Sn, as a result the central Sn_2O_2 core is fused with two four-membered (Sn(1)–O(1)–Sn(2)–O(13)) and two six-membered (Sn(1)–O(13)–Sn(2)–O(8)–C(9)–O(7)) rings. The endocyclic Sn(2) is six coordinated in a skew trapezoidal bipyramidal environment while exocyclic Sn(1) is five coordinated with distorted trigonal bipyramidal geometry. The ligand acid and synthesized organotin compounds were also screened for antibacterial, antifungal, brine-shrimp lethality and potato disc antitumor activities. Results of bioassay demonstrate that organotin derivatives are in general more active than the ligand acid.

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1. Introduction

Organotin(IV) carboxylates are being extensively studied with special reference to their methods of synthesis, structural elucidation and biological activities [1–7]. Generally these compounds are well characterized by FT-IR, multinuclear NMR (^1H, ^{13}C, ^{119}Sn), X-ray and ^{119m}Sn Mössbauer spectroscopy [8–12]. In addition extensive biocidal applications utilizing the antitumor and anticancer activities of such compounds has made them significantly important [5,6]. Another aspect of major interest in organotin carboxylates is of their structural diversity. Both diorganotin(IV) and triorganotin(IV) esters show rich and diverse structural chemistry as citied in recent reviews [7,9].

Piperonylic acid is a natural molecule bearing a methelenedioxy function that closely mimics the structure of *trans*-cinnamic acid [13a] and possess antimicrobial properties [13b]. It is extracted from the bark of Paracoto tree. It can also be prepared in laboratory by the oxidation of piperonal with $KMnO_4$ [13c]. To the best of our knowledge no metal complexes has been reported for Piperonylic acid until we reported first time organotin(IV) complexes of piperonylic acid with structural and biological activities [14]. On the basis of our previous investigation on structural and biological activities organotin(IV) complexes of piperonylic acid [14–18], we extended our work to synthesis organotin(IV) derivatives of 6-nitropiperonylic acid (Fig. 1) and to establish their biological role [14–18].

2. Experimental

2.1. Materials

All the organotin precursors and chemicals used were procured from Aldrich or Fluka. All the solvents were dried before use by the literature methods [19].

2.2. Instrumentation

Melting points were determined in a capillary tube using a MPD Mitamura Riken Kogyo (Japan) electrothermal melting point

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doi:10.1016/j.poly.2009.07.039

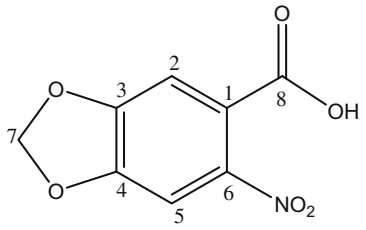

Fig. 1. Numbering scheme and structure of the 6-nitropiperonylic acid (HL).

apparatus and are uncorrected. Multinuclear NMR (^{1}H, ^{13}C and ^{119}Sn) spectra were recorded on a Bruker ARX 300 MHz-FT-NMR spectrometer using $CDCl_3$ as an internal reference for [δ ^{1}H ($CDCl_3$) = 7.25 and δ ^{13}C ($CDCl_3$) = 77.0]. ^{119}Sn NMR spectra were obtained with Me_4Sn as an external reference [(^{119}Sn) = 37.290665]. Chemical shifts (δ) are given in ppm and coupling constants *J* are given in Hz. The multiplicities of signals in ^{1}H NMR are given with chemical shifts; (s = singlet, t = triplet, q = quartet, m = multiplet).

2.3. Synthesis

2.3.1. Synthesis of the ligand LH

The ligand acid was synthesized by the method reported elsewhere [14]. In a typical procedure 6-nitropiperonal (25.7 g, 1.32 mmol) and 41.6 g (2.64 mmol) of potassium permanganate were added in a mixture of water (300 mL) and acetone (200 mL). The mixture was mechanically stirred and reflux for 4 h. After reflux acetone was distilled off, the flask was cooled and MnO_2 precipitate was filtered off, washed with 100 mL (in two 50 mL portions) of hot water. The filtrate was ice cooled in an ice bath and acidified to produce analytically pure yellow solid. M.p. 166–167 °C. Yield 68%.

2.3.2. Synthesis of the ligand NaL

The sodium salt of the ligand acid was prepared by adding drop wise an equimolar amount of sodium hydrogen carbonate dissolved in distilled water to the ligand acid dissolved in ethanol. The clear solution obtained was concentrated under reduced pressure. The solid obtained was dried over CaO/P_2O_5.

2.3.3. Synthesis of organotin derivatives

2.3.3.1. General procedure. Two different methods have been employed for the synthesis of the organotin derivatives of the 6-nitropiperonylic acid. In method A the organotin chloride was refluxed with sodium salt of the acid in dry toluene for 5–6 h in 1:2 (diorganotin dichloride) or 1:1 (triorganotin chloride) molar ratio. After reflux the insoluble material were filtered off and solvent was evaporated under reduce pressure. The resultant solid masses were recrystallized from chloroform and *n*-hexane mixture. In method B appropriate R_2SnO and ligand were refluxed for 6 h in 1:2 molar ratios in dry toluene (100 mL) using Dean-stark apparatus for azeotropic removal of water formed during the condensation reaction. The reaction mixture was then cooled to room temperature and the solvent was rotary evaporated. The solid product obtained was recrystallized from a mixture of chloroform and *n*-hexane.

*2.3.3.1.1. Compound (**1**) Me_2SnL_2.* Prepared by method A by adding sodium salt of ligand (1 g, 4.29 mmol) and Me_2SnCl_2 (0.47 g, 2.15 mmol) in 2:1 ratio, respectively.

Physical data; Yield 82%, m.p. 229–231 °C, CHN *Anal.* Calc.: C, 37.96; H, 2.46; N, 4.92. Found: C, 38.06; H, 2.51; N, 4.86%.

FT-IR data (KBr, cm^{-1}); $\upsilon(COO)_{Asym}$ 1602, $\upsilon(COO)_{sym}$ 1412, $\Delta\upsilon$ 190, υ(Sn–C) 577, υ(Sn–O) 445.

^{1}H NMR data ($CDCl_3$, ppm, ^2J[^{119}Sn,^{1}H] in Hz); 7.46 (s, 2H, aromatic protons), 7.25 (s, 2H, aromatic protons), 6.31 (s, 4H, O–CH_2–O), {1.26 (s, 6H, [79], $SnCH_3$)}.

^{13}C NMR data ($CDCl_3$, ppm); 129.0 (C1), 108.5 (C2), 151.3 (C3), 149.8 (C4), 105.0 (C5), 143.2 (C6), 103.6 (C7), 174.3 (C8), {4.6 ($SnCH_3$)}.

^{119}Sn NMR data ($CDCl_3$, ppm); −172.0.

*2.3.3.1.2. Compound (**2**) Me_3SnL.* Prepared by method A by adding sodium salt of ligand (1 g, 4.29 mmol) and Me_3SnCl (0.86 g, 4.29 mmol) in 1:1 ratio, respectively.

Physical data; Yield 76%, m.p. 188–191 °C, CHN *Anal.* Calc.: C, 35.36; H, 3.48; N, 3.74. Found: C, 35.24; H, 3.51; N, 3.65%.

FT-IR data (KBr, cm^{-1}); $\upsilon(COO)_{Asym}$ 1624, $\upsilon(COO)_{sym}$ 1424, $\Delta\upsilon$ 200, υ(Sn–C) 550, υ(Sn–O) 450.

^{1}H NMR data ($CDCl_3$, ppm, ^2J[^{119}Sn, ^{1}H] in Hz); 7.33 (s, 1H, aromatic protons), 7.26 (s, 1H, aromatic protons), 6.13 (s, 2H, O–CH_2–O), {1.25 (s, 9H, [57], $SnCH_3$)}.

^{13}C NMR data ($CDCl_3$, ppm, ^1J[$^{117/119}$Sn, ^{13}C] in Hz); 128.5 (C1), 110.7 (C2), 153.4 (C3), 150.2 (C4), 106.9 (C5), 144.4 (C6), 105.6 (C7), 173.6 (C8), {0.1 ($SnCH_3$, [388, 400])}.

^{119}Sn NMR data ($CDCl_3$, ppm); 128.5.

*2.3.3.1.3. Compound (**3**) Et_2SnL_2.* Prepared by method A by adding sodium salt of ligand (1 g, 4.29 mmol) and Et_2SnCl_2 (0.53 g, 2.15 mmol) in 2:1 ratio, respectively.

Physical data; Yield 80%, m.p. 200–202 °C, CHN *Anal.* Calc.: C, 40.2; H, 3.02; N, 4.69. Found: C, 40.13; H, 2.961; N, 4.53%.

FT-IR data (KBr, cm^{-1}); $\upsilon(COO)_{Asym}$ 1610, $\upsilon(COO)_{sym}$ 1414, $\Delta\upsilon$ 196, υ(Sn–C) 545, υ(Sn–O) 465.

^{1}H NMR data ($CDCl_3$, ppm, ^nJ(^{1}H, ^{1}H), ^2J[^{119}Sn, ^{1}H] in Hz); 7.36 (s, 2H, aromatic protons), 7.16 (s, 2H, aromatic protons), 6.19 (s, 4H, O–CH_2–O), {1.94 (q, 4H, (8.1), [75]), 1.47 (t, 6H, (8.0), $SnCH_2CH_3$)}.

^{13}C NMR data ($CDCl_3$, ppm, ^nJ[^{119}Sn, ^{13}C] in Hz); 129.1 (C1), 108.8 (C2), 151.0 (C3), 149.6 (C4), 104.9 (C5), 143.4 (C6), 103.5 (C7), 174.4 (C8), {17.7 [580], 9.0 [43] ($SnCH_2CH_3$)}.

^{119}Sn NMR data ($CDCl_3$, ppm); −157.7.

*2.3.3.1.4. Compound (**4**) $[(n\text{-}Bu_2SnL)_2O]_2{\cdot}CHCl_3$.* Prepared by method B by adding ligand acid (1 g, 4.74 mmol) and *n*-Bu_2SnO (0.59 g, 2.37 mmol) in 2:1 ratio, respectively.

Physical data; Yield 65%, m.p. 196–198 °C, CHN *Anal.* Calc.: C, 38.77; H, 4.4; N, 2.74. Found: C, 38.02; H, 4.22; N, 2.62%.

FT-IR data (KBr, cm^{-1}); $\upsilon(COO)_{Asym}$ 1609, $\upsilon(COO)_{sym}$ 1420, $\Delta\upsilon$ 189, υ(Sn–O-Sn–O) 686, υ(Sn–C) 563, υ(Sn–O) 445.

^{1}H NMR data ($CDCl_3$, ppm, ^nJ(^{1}H, ^{1}H), ^2J[^{119}Sn, ^{1}H] in Hz); 7.3 (s, 4H, aromatic protons), 6.91 (s, 4H, aromatic protons), 6.15 (s, 8H, O–CH_2–O), {1.82 (t, 16H, (8), [73]), 1.51–1.38 (m, 32H), 0.91 (t, 24H, (7.4), $SnCH_2CH_2CH_2CH_3$)}.

^{13}C NMR data ($CDCl_3$, ppm); 127.2 (C1), 107.8 (C2), 151.1 (C3), 148.8 (C4), 104.6 (C5), 142.3 (C6), 103.3 (C7), 170.2 (C8), {26.8, 27.4, 27.8, 13.7 ($SnCH_2CH_2CH_2CH_3$)}.

^{119}Sn NMR data ($CDCl_3$, ppm); −198.2, −199.8.

*2.3.3.1.5. Compound (**5**) n-Oct_2SnL_2.* Prepared by method B by adding ligand acid (1 g, 4.74 mmol) and *n*-Oct_2SnO (0.86 g, 2.37 mmol) in 2:1 ratio, respectively.

Physical data; Yield 77%, m.p. 134–137 °C, CHN *Anal.* Calc.: C, 50.19; H, 5.49; N, 3.66. Found: C, 50.09; H, 5.40; N, 3.86%.

FT-IR data (KBr, cm^{-1}); $\upsilon(COO)_{Asym}$ 1619, $\upsilon(COO)_{sym}$ 1427, $\Delta\upsilon$ 192, υ(Sn–C) 564, υ(Sn–O) 480.

^{1}H NMR data ($CDCl_3$, ppm, ^nJ(^{1}H, ^{1}H) in Hz); 7.45 (s, 2H, aromatic protons), 6.88 (s, 2H, aromatic protons), 6.04 (s, 4H, O–CH_2–O), {1.65–1.1 (m, 28H), 0.86 (t, 6H, (8), Sn $CH_2CH_2CH_2CH_2$-$CH_2CH_2CH_2CH_3$)}.

^{13}C NMR data ($CDCl_3$, ppm); 127.1 (C1), 107.9 (C2), 151.0 (C3), 148.8 (C4), 104.6 (C5), 142.4 (C6), 103.3 (C7), 170.2 (C8), {25.3, 22.7, 34.1, 29.5, 29.4, 32.0, 25.5, 14.1, ($SnCH_2CH_2CH_2CH_2$-$CH_2CH_2CH_2CH_3$)}.

^{119}Sn NMR data ($CDCl_3$, ppm); −199.1.

*2.3.3.1.6. Compound (**6**) Ph_3SnL.* Prepared by method B by adding ligand acid (1 g, 4.74 mmol) and Ph_3SnOH (1.74 g, 4.74 mmol) in 1:1 ratio, respectively.

Physical data; Yield 83%, m.p. >300 °C, CHN *Anal.* Calc.: C, 55.71; H, 3.4; N, 2.50. Found: C, 55.63; H, 3.28; N, 2.51%.

FT-IR data (KBr, cm^{-1}); $\upsilon(COO)_{Asym}$ 1608, $\upsilon(COO)_{sym}$ 1413, $\Delta\upsilon$ 195, υ(Sn–O) 449.

^1H NMR data ($CDCl_3$, ppm); {7.73–7.49 (m, 15H, SnPh)}, 7.31 (s, 1H, aromatic protons), 7.26 (s, 1H, aromatic protons), 6.27 (s, 2H, O–CH_2–O).

^{13}C NMR data ($CDCl_3$, ppm); 128.9 (C1), 109.2 (C2), 150.6 (C3), 149.4 (C4), 104.6 (C5), 137.5 (C6), 103.3 (C7), 173.1 (C8), {129.6, 136.9, 130.4, 129.3, (SnPh)}.

^{119}Sn NMR data ($CDCl_3$, ppm); −125.1.

2.4. X-ray crystallography

All X-ray crystallographic data were collected on a Stoe Imaging Plate Diffractometer System. Correction for semi-empirical from equivalents was applied, and the structure was solved by direct methods and refined by a full-matrix least squares procedure based on F^2 using the SHELXS-97 and SHELXL-97 Program System [20]. All data were collected with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) at 173 K. Table 1 present the crystallographic data for the compound **4**.

2.5. Biological activity

2.5.1. Antibacterial assay

All these synthesized compounds and their acid were tested against six bacterial strains; three Gram-Positive [*Bacillus subtilis* (ATCC6633), *Micrococcus luteus* (ATCC10240) and *Staphylococcus aureus* (ATCC6538)] and three Gram-negative [*Escherichia coli* (ATCC15224), *Enterobactor aerogenes* (ATCC13048), *Bordetella bronchiseptica* (ATCC4617)]. The agar well-diffusion method was used for the determination of inhibition zones and minimum inhibitory concentration (MIC) [21]. Briefly 0.75 mL of the broth culture containing ca. 10^6 colony forming units (CFU) per mL of the test strain was added to the 75 mL of nutrient agar medium at 45 °C, mixed well, and then poured into a 14 cm sterile petri plate. The media was allowed to solidify, and 8 mm wells were dug with a sterile metallic borer. Then a DMSO solution of test sample (100 μL) at 1 mg/mL was added to the respective wells. DMSO served as negative control, and the standard antibacterial drugs Roxyithromycin (1 mg/mL) and Cefixime (1 mg/mL) were used as positive control. Triplicate plate of each bacterial strain was prepared. The plates were incubated aerobically at 37 °C for 24 h. The activity was determined by measuring the diameter of zone showing complete inhibition (mm). Thereby zones were precisely measured with the aid of a vernier caliper (precision ± 0.1 mm). The growth inhibition was calculated with reference to the positive control. For individual compounds that showed inhibition >10 mm, MIC values were determined by using agar well-diffusion method [21].

Table 1
Crystal Data and structure refinements for compound **4**.

	Compound **4**
Empirical formula	$C_{32}H_{44}N_2O_{13}Sn_2{\cdot}CHCl_3$
Formula weight	1021.44
Crystal shape (color)	block (yellow)
Crystal system	triclinic
Space group	$P\bar{1}$
Unit cell dimension	
a(Å)	12.3636(14)
b (Å)	13.0105(16)
c (Å)	13.4017(15)
α (°)	90.164(14)
β (°)	98.435(13)
γ (°)	100.292(14)
V (Å ^{3})	2097.2(4)
Z	2
D_{calc} (g cm^{-3})	1.618
Crystal size (mm)	0.50 × 0.50 × 0.50
F(0 0 0)	1024
Total reflections	15 415
Independent reflections	7458
Observed reflections	6084
All indices (all data)	R_1 = 0.0672
	wR_2 = 0.1802
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0818
	wR_2 = 0.1984
Goodness-of-fit	1.054
θ Range for data collection (°)	2.4–25.8

2.5.2. Antifungal assay

Antifungal activity against six fungal strains [*Fusarium moniliformis, Alternaria* specie, *Aspergillus niger*, *Fusarium solani*, *Mucor specie* and *Aspergillus fumigatus*] was determined by using Agar tube dilution method [21]. Screw caped test tubes containing Sabouraud dextrose agar (SDA) medium (4 mL) were autoclaved at 121 °C for 15 min. Tubes were allowed to cool at 50 °C and non solidified SDA was loaded with 66.6 μL of compound from the stock solution (12 mg/mL in DMSO) to make 200 μL/mL final concentration. Tubes were then allowed to solidify in slanting position at room temperature. Each tube was inoculated with 4 mm diameter piece of inoculum from seven days old fungal culture. The media supplemented with DMSO and Turbinafine (200 μL/mL) were used as negative and positive control, respectively. The tubes were incubated at 28 °C for 7 days and growth was determined by measuring linear growth (mm) and growth inhibition was calculated with reference to the negative control.

2.5.3. Cytotoxicity

The cytotoxicity was studied by the brine-shrimp lethality assay method [21,22]. Brine-shrimp (*Artemia salina*) eggs were hatched in artificial sea water (3.8 g sea salt/L) at room temperature (22–29 °C). After two days these shrimps were transferred to vials containing 5 mL of artificial sea water (30 shrimps per vial) with 10 100 and 1000 ppm final concentrations of each compound taken from their stock solutions of 12 mg/mL in DMSO. After 24 h number of surviving shrimps was counted. Data was analyzed with a finny computer programme (Probit analysis) to determine LD_{50} values.

2.5.4. Antitumor activity

Antitumor Potato Disc Assay [21] was also performed for all these synthesized compounds. Potato discs (0.5 cm thickness) were obtained from surface sterilized potatoes by using metallic cork borer and special cutter under complete aseptic conditions. These potato discs were then transferred Petri plates each containing 25 mL of 1.5% agar solution. Then 0.5 mL of stock (10 mg/mL) of the test sample was added to 2 mL of a broth culture of *Agrobacterium tumefaciens* (At10, a 48 h culture containing 5×10^9 cells/mL) and 2.5 mL of autoclaved distilled water was added to make 1000 ppm concentration. One drop of these cultures was poured on each potato disc. The petri plates were incubated at 28 °C. After 21 days incubation, the number of tumors was counted with the aid of dissecting microscope after staining with Lugol's solution.

3. Result and discussion

The synthesis of organotin derivatives of 6-nitropiperonylic acid may be represented by the following equations:

$$R_3SnCl + \underset{R=Me(\mathbf{2})}{NaL} \rightarrow R_3SnL + NaCl$$

$$R_2SnCl_2 + \underset{R=Me(\mathbf{1}),Et(\mathbf{3})}{2NaL} \rightarrow R_2SnL_2 + 2NaCl$$

$$R_2SnO + \underset{R=Oct(\mathbf{5})}{2LH} \rightarrow R_2SnL_2 + H_2O$$

$$R_3SnOH + \underset{R=Ph(\mathbf{6})}{LH} \rightarrow R_3SnL + H_2O$$

In an attempt to prepare diorganotin dicarboxylate with general formula Bu_2SnL_2, only the compound $\{[(LSnBu_2]_2O\}_2$ **4** was isolated after recrystallization as evident by the observed two ^{119}Sn NMR resonances of almost equal intensity and X-ray crystal structure. Apparently, either only one equivalent of acid can be consumed or the diorganotin dicarboxylates formed can undergo a hydrolysis to yield the dimeric distannoxanes as shown below [23].

$$4Bu_2SnO + 4LH \rightarrow \underset{(\mathbf{4})}{\{(Bu_2SnL)_2O\}_2} + 2H_2O$$

$$4Bu_2SnL_2 + 2H_2O \rightarrow \underset{(\mathbf{4})}{\{(Bu_2SnL)_2O\}_2} + 4LH$$

All the newly synthesized compounds are air stable, crystalline solids and soluble in common organic solvents. These newly synthesized compounds were characterized by FT-IR, and multinuclear NMR (^{1}H, ^{13}C and ^{119}Sn) spectroscopy. Further single crystal X-ray structure of compound **4** is also determined.

3.1. Infrared spectroscopy

The absorption frequencies of interest are υ(COO), υ(Sn–C) and υ(Sn–O). The absence of a broad band in the range 3100–2500 cm^{-1} appearing in the ligand acid, as υ(OH) vibrations, indicating metal–ligand bond formation through this site. Similarly, absorption bands in the range 577–545 and 485–445 cm^{-1}, assigned to Sn–C and Sn–O bonds, respectively, also support the formation of complexes [24]. Bands due to nitro group are also observed in the complexes at the same frequencies as observed in the free ligand, indicating that nitro group is not coordinated to tin(IV). The shift in υ(COO) vibrations to lower frequencies as compare to ligand acid and magnitude of $\Delta\upsilon$(COO) in the range of 189–200 cm^{-1} indicating a bidentate nature of the carboxylate towards the Sn atom [25]. Thus according to the earlier reports featuring the same results and crystallographic data [14,18], it is most likely that in diorganotin(IV) compounds, the tin atom approaches six coordination based on skew trapezoidal planar geometry and the carboxylate group acts as chelating bidentate ligand while in triorganotin(IV) compounds, the tin atom approaches five coordination and carboxylate group acts as bridiging bidentate ligand leading to trigonal bipyramidal with *trans*-R_3SnO_2 geometry [25]. The IR spectrum of compound **4** is almost similar to diorganotin compounds except for a very sharp band at 686 cm^{-1}, characteristic for Sn–O–Sn–O four-membered rings as dimeric dicarboxylatotetraorgano distannoxane [23].

3.2. Multinuclear (^{1}H, ^{13}C, ^{119}Sn) NMR spectroscopy

Multinuclear NMR spectra (^{1}H, ^{13}C, ^{119}Sn) for new compounds and free acid have been recorded in $CDCl_3$ and DMSO-d_6 solution, respectively, and are given in experimental part along the synthesis of compounds. The single resonance at 10 ppm due to carboxylic acid proton is absent in the ^{1}H NMR spectra of the complexes indicating the replacement of the carboxylic acid proton with organotin(IV) moieties. In the spectrum of ligand acid the methylenedioxy protons (O–CH_2–O) at 6.33 ppm and aromatic protons at 7.37 and 7.66 ppm are appeared as singlet and a set of similar pattern has been observed in the complexes. The methyl protons in dimethyltin **1** and trimethyltin **2** derivatives appear as sharp singlet with $^2J[^{119}Sn,^1H]$ coupling of 79 and 57 Hz, respectively. The protons of $SnCH_2CH_3$ group in 3 appear at 1.94 and 1.47 ppm with distinct quartet and triplet, respectively. Both methylene and methyl protons show well resolved $^3J[^1H,^1H]$ couplings and methylene protons show $^2J[^{119}Sn,^1H]$ coupling of 75 Hz. In complex **4** the protons of *n*-butyl group show complex pattern due to –CH_2–CH_2– skeleton in the range 1.38–1.51 ppm and clear triplet for Sn–CH_2 (at 1.82 ppm) and terminal CH_3 (at 0.91 ppm) with $^2J[^{119}Sn,^1H]$ and $^3J[^1H,^1H]$ couplings. The methylene protons $(CH_2)_7$ *n*-octyl moiety of **5** exhibit a somewhat different behavior and appear as broad signals in the range 1.1–1.65 ppm comparable with analogues compounds [14]. However, terminal CH_3 protons give a triplet at 0.86 ppm with $^3J[^1H,^1H]$ coupling of 8 Hz. A complex pattern for aromatic protons in case of triphenyltin **6** has also been observed in the expected region.

^{13}C NMR spectral data for ligand acid and its organotin(IV) derivatives are listed along their synthesis in the experimental section. The assignment of ^{13}C signals for ligand and its organotin derivatives are assigned by comparison with related compounds, by results obtained from incremental methods and by $^nJ[^{119}Sn,^{13}C]$ couplings [26]. The chemical shift of carboxylate carbon shifts to a lower field region in almost all the organotin(IV) derivatives indicating participation of the carboxyl (COO) group in coordination to tin atom [14]. The chemical shift of the methylene and aromatic carbons undergo minor variation in the complexes, as compare to those observed in the free acid. The organic moiety attached to tin atom display the expected carbon signals [27]. In addition $^nJ[^{119}Sn,^{13}C]$ couplings are also observed in some complexes. $^nJ[^{119}Sn,^{13}C]$ satellites are important indicators for structural evaluation of organotin carboxylates. Holecek and coworkers have shown that for four coordinated trialkyltin and triphenyltin compounds, the coupling constants $^1J[^{119}Sn,^{13}C]$ occur in the range of 325–400 and 550–670 Hz, respectively, while five coordinated tin compounds exhibit couplings in the range of 440–540 and 750–850 Hz, respectively. In the present investigation the trimethyltin **2** compound show tin-carbon satellite of 400 Hz in solution ($CDCl_3$), characteristic of the tetrahedral geometry around tin atom. Thus bidentate nature of the ligand acid resulting in penta-coordination in solid state (FT-IR) is therefore lost in solution to generate a monomer four coordinated tetrahedral structure for **2** [28]. Unfortunately we are not able to record the tin–carbon satellite for triphenyltin derivative. In diorganotin compounds, $^1J[^{119}Sn,^{13}C]$ satellites are observed for diethyltin **3** compound, which suggest coordination number higher than four in comparison with the reported values [29]. However, it is not defined with certainty because of the fluxional behavior of the carboxylate oxygen in their coordination with the tin atom but most of alkyl diorganotin(IV) carboxylates appear as skew trapezoidal geometry which is in between five and six coordination numbers [30]. We emphasized, therefore, on the ^{119}Sn NMR (see synthesis part in Section 2) in order to deduce the coordination of tin atom in diorganotin compounds as well as triorganotin compounds. Compounds **1**, **3** and **5** exhibit a single ^{119}Sn chemical shift at −172.0, 157.7 and −199.1 ppm, respectively, characteristic of penta-coordinated tin atom as earlier reports manifested [14–18]. On the other hand trimethyltin **2** give a single resonance peak in the range characteristic for tetrahedral compounds. Triphenyltin **6** show ^{119}Sn chemical shift at −125.1 ppm which is very close to the reported four coordinated triphenyltin compounds and less than the maximum value of −128.1 ppm for four coordinated tetraphenyltin compound [27]. For compound **4** two ^{119}Sn resonances of almost equal intensities are observed (−198.2 and −199.8 ppm) and assigned to the five coordinated endocyclic and exocyclic tin atoms. These values are in agreement with the literature values reports for similar distannoxanes [23,31].

Various literature methods have been applied to calculate C–Sn–C bond angles in solution, based on $^2J[^{119}Sn,^1H]$ and

$^1J[^{119}Sn,^{13}C]$ coupling constants [32,33]. By the use of Lockhart and Holecek equations, the values obtained are 111.8° and 127.6° for compounds **2** and **3**, respectively on the basis of $^1J[^{119}Sn,^{13}C]$ couplings. The geometrical data calculated are consistent with tetrahedral and skew trapezoidal geometry, respectively, for tri- and diorganotin compounds.

3.3. Crystal structure of *4*

The molecular structure of **4** is shown in Fig. 2 and selected interatomic parameters are given in Table 2. The compound **4** is crystallized with a solvent molecule of $CHCl_3$ which is disordered over two orientations with site occupancy ratio of 0.5:0.5. The structure of **4** is composed of a tetranuclear centrosymmetric dimer of oxoditin unit with a central four-membered ring defined by Sn(2)–O(13)–Sn(2)^i–O(13)^i (i symmetry transformations used to generate equivalent atoms: $-x, -y+1, -z$). This central Sn_2O_2 core is fused with two four-membered (Sn_2O_2, i.e., O(13)–Sn(1)–O(1)–Sn(2)^i) and two six-membered (Sn_2O_3C, i.e., Sn(1)–O(13)–Sn(2)–O(8)–C(9)–O(7)) rings. The each endocyclic Sn(2) atom of the central Sn_2O_2 core is six coordinated in C_2SnO_4 skew trapezoidal bipyramidal geometry, wherein Sn(2) is coordinated to O(8) of bidentate ligand acid anion bridging endocyclic Sn(2) with exocyclic Sn(1) through O(8)–C(9)–O(7), two α-carbon atoms of *n*-butyl groups and O(1) of monodenate ligand acid also bridging Sn(1) forming four-membered ring. The bidentate bridging ligand forms almost equivalent interactions with tin, i.e., Sn(2)–O(8) = 2.287(6) and Sn(1)–O(7) = 2.286(6) Å. The other endocyclic Sn–O distances in the Sn_2O_2 core, Sn(2)–O(13) = 2.046(5) and Sn(2)–O(13)^i = 2.155(5) Å, are quite close to other related compounds [34]. The sixth position of the skew trapezoidal geometry is occupied by Sn(2)–O(1) bond with Sn–O distance Sn(2)–O(1) = 2.722(6) Å. This indicates a quite long and weaker Sn–O interaction. This longer Sn–O bond is much longer than the sum of the covalent radii of the tin and oxygen (2.13 Å) but significantly below the sum of the van der Waal's radii of these atoms (3.68 Å) [14].

It is worth noted that some reports do not consider such long interactions as significant bonding contacts [35,36]. However, such intramolecular Sn–O contacts in the range of 2.61–3.02 Å have been confidently reported for intramolecular Sn–O bonding. In this case, the O(1) acts as monoatomic bridging atom (μ_2) and connect the endocyclic and exocyclic tin centers thus making endocyclic Sn(2) six coordinated [37]. However the distance Sn(1)–O(2) = 3.020(6) Å is considered to be very weak intramolecular interaction when compare to Sn(2)–O(1) distance and could not be considered for any coordination with Sn(1) [9]. The axial C–Sn–C angle, C(25)–Sn(2)–C(29) = 144.9(4)° is distorted from ideal value of 180° by nearly 35° so as to better occupy the open space left by the skew trapezoidal arrangement of the equatorial ligands [37].

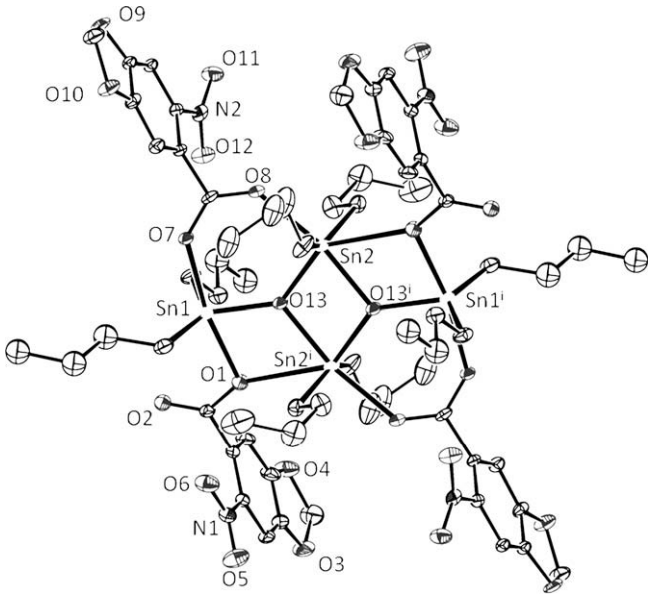

Fig. 2. Molecular structure of compound **4**. The displacement ellipsoids are drawn at the 30% probability level. Atoms labeled with a superscript (i) are related to the others by an inversion center (hydrogen atoms and solvent molecules have been removed for clarity).

Table 2
Selected bond lengths (Å) and bond angles (°) for compound **4**.[a]

Bond lengths (Å)			
C(1)–O(2)	1.218(11)	C(1)–O(1)	1.321(10)
C(9)–O(8)	1.242(10)	C(9)–O(7)	1.272(10)
C(17)–Sn(1)	2.143(9)	C(21)–Sn(1)	2.116(9)
C(25)–Sn(2)	2.121(9)	C(29)–Sn(2)	2.125(8)
O(1)–Sn(1)	2.190(5)	O(7)–Sn(1)	2.286(6)
O(8)–Sn(2)	2.287(6)	O(13)–Sn(1)	2.038(5)
O(13)–Sn(2)	2.046(5)	O(13)–Sn(2)^i	2.155(5)
O(13)^i–Sn(2)	2.155(5)		
Bond angles (°)			
O(2)–C(1)–O(1)	123.7(7)	O(8)–C(9)–O(7)	126.5(7)
O(13)–Sn(1)–C(21)	114.3(3)	O(13)–Sn(1)–C(17)	108.7(3)
C(21)–Sn(1)–C(17)	136.5(4)	O(13)–Sn(1)–O(1)	77.78(19)
C(21)–Sn(1)–O(1)	96.5(3)	C(17)–Sn(1)–O(1)	98.8(3)
O(13)–Sn(1)–O(7)	91.0(2)	C(21)–Sn(1)–O(7)	85.3(3)
C(17)–Sn(1)–O(7)	87.6(3)	O(1)–Sn(1)–O(7)	168.4(2)
O(13)–Sn(2)–C(25)	103.9(4)	O(13)–Sn(2)–C(29)	110.4(3)
C(25)–Sn(2)–C(29)	144.9(4)	O(13)–Sn(2)–O(13)^i	75.7(2)
C(25)–Sn(2)–O(13)^i	99.7(3)	C(29)–Sn(2)–O(13)^i	95.8(3)
O(13)–Sn(2)–O(8)	89.8(2)	C(25)–Sn(2)–O(8)	88.3(3)
C(29)–Sn(2)–O(8)	84.6(3)	O(13)^i–Sn(2)–O(8)	164.7(2)
Sn(1)–O(13)–Sn(2)	134.8(2)	Sn(1)–O(13)–Sn(2)^i	120.3(2)
Sn(2)–O(13)–Sn(2)^i	104.3(2)		

[a] Symmetry transformations used to generate equivalent atoms: i $-x, -y+1, -z$.

The exocyclic Sn(1) atoms are five coordinated and show distorted trigonal bipyramidal geometry. Sn(1) atom is coordinated with O(7) of bidentate briding ligand anion, O(1) of monatomic briding ligand, O(13) of central Sn_2O_2 core and two α-carbon atoms of *n*-butyl groups. The axial C–Sn–C angle, C(17)–Sn(2)–C(21) = 136.5(4)°.

The Sn–C distances, which are almost identical, lie within the narrow range of 2.116(9)–2.143(9) Å and are in agreement with the values reported for related structures [9]. It is interesting to note that one of the ligand acid is coordinated to both Sn atoms with C–O distances, C(9)–O(7) = 1.272(10) and C(9)–O(8) = 1.242(10) Å, and lying between a single and double bond representing a delocalized system. In other ligand the C–O distances, C(1)–O(1) = 1.321(10) and C(1)–O(2) = 1.218(11) Å, clearly indicate a single and a double bond.

The ligand acid shows different modes of coordination with tin. It acts as a monodentate bridging ligand, bridging the two exo and endo Sn atoms via O(1) forming four-membered rings. The ligand also bridges two Sn atoms in bidentate coordination mode via O(7) and O(8) thus resulting in six-membered rings.

3.4. Biological activity

In vitro biocidal screening tests of synthesized organotin compounds and their acid were carried out for antibacterial, antifungal, cytotoxicity and antitumor activity. The antibacterial activity was tested against six bacterial strains; three Gram-positive [*S. aureus* (ATCC6538), *B. subtilis* (ATCC6633), *M. luteus* (ATCC10240)] and three Gram-negative [*E. aerogenes* (ATCC13048), *E. coli*

(ATCC15224) and *B. bronchiseptica* (ATCC4617)]. The agar well-diffusion method [21] was used in these assays and each experiment was performed in triplicate. Readings of the zone of inhibition represents the mean value of three readings with standard deviation (STDEV), which are shown in Table 3. Roxyithromycin and Cefixime were used as standard drugs in these assays. Criteria for activity is based on inhibition zone (mm); inhibition zone more than 12 mm shows significant activity, for 10–12 mm inhibition activity is good, 7–9 mm is low, and below 7 mm is non-significant activity. The antibacterial studies demonstrated that all complexes have activity toward tested bacteria than their ligand acid which show no activity [13,14]. The values obtained for activity for compounds **1**–**4** and **6** falls in criteria of significant activity except for compound **5** which show low activity against two pathogenic strains, *S. aureus* and *M. luteus* and non-significant activity against the rest four pathogenic strains. Compound **6** (triphenyltin derivative) have the highest activity among all synthesized compounds and comparable with one of the reference drug Roxyithromycin. Compound **4** also show non-significant activity against *B. subtilis.*

All synthesized compounds were also subjected to antifungal activity against six fungal strains *[F. moniliforme*, *Alternaria* species, *A. niger*, *F. solani*, *Mucor* species and *A. fumigatus*] by using Agar tube dilution method [21]. The results are shown in Table 4. Terbinafine was used as standard drug in this assay. Criteria for activity is based on percent growth inhibition; more than 70% growth inhibition shows significant activity, 60–70% inhibition activity is good, 50–60% inhibition activity is moderate and below 50% inhibition activity is non-significant. The investigation shows that all the synthesized organotin compounds in general have more activity than the parent acid except few cases but less than the reference drug Terbinafine. Compound **4** show 100% growth inhibition against *F. moniliforme*. Ligand acid and compounds **1** and **5** show no activity against some fungal strains.

The cytotoxicity was studied by the brine-shrimp bioassay lethality method [21,22] and the results are summarized in Table 5. The LD_{50} data show that all the tested compounds even the ligand acid are toxic with LD_{50} values in the range 0–230.78 μg/mL in comparison to reference drug MS-222 (Tricaine Methanesulfonate) with LD_{50} value 4.3 μg/mL. Triphenyltin **6** and distannoxane **4** are the most toxic as compare to tested compounds and ligand acid.

Table 3
Antibacterial activities of 6-nitropiperonylic acid and its organotin(IV) derivatives.[a]

Compound no.	Mean zone of inhibition (mm) ± SD					
	S. aureus (ATCC6538)	*B. subtillus* (ATCC6633)	*M. luteus* (ATCC10240)	*E. aerogenase* (ATCC13048)	*E. coli* (ATCC5224)	*B. bronchiseptica* (ATCC4617)
1	22.3 ± 0.20	22.1 ± 0.23	20.8 ± 0.37	21.3 ± 0.34	19.2 ± 0.70	22.4 ± 0.45
2	20.6 ± 0.15	22.4 ± 0.66	21.7 ± 0.05	18.4 ± 0.13	19.8 ± 0.15	23.5 ± 0.98
3	21.0 ± 0.28	20.8 ± 0.23	18.6 ± 0.20	22.3 ± 0.38	21.0 ± 00	20.6 ± 0.07
4	20.0 ± 0.39	1.88 ± 0.23	12.6 ± 0.14	21.3 ± 0.26	18.5 ± 0.20	20.2 ± 0.30
5	8.5 ± 0.28	2.4 ± 0.66	11.6 ± 0.23	3.1 ± 0.45	5.7 ± 0.12	2.7 ± 0.40
6	23.5 ± 0.37	24.0 ± 0.00	23.3 ± 0.20	24.4 ± 0.26	24.0 ± 0.00	24.2 ± 0.10
HL	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00
Roxyithromycin	23.5 ± 0.50	24.0 ± 0.00	23.3 ± 0.26	24.4 ± 0.05	24.33 ± 0.20	24.2 ± 0.25
Cefixime	27.2 ± 0.70	27.6 ± 0.75	22.7 ± 0.30	25.3 ± 0.45	27.6 ± 0.00	27.8 ± 0.56

[a] *In vitro*, agar well-diffusion method, concentration: 1 mg/mL of DMSO, reference drugs, Roxyithromycin and Cefixime, concentration: 1 mg/mL of DMSO.

Table 4
Antifungal activities of 6-nitropiperonylic acid and its organotin(IV) derivatives.[a]

Compound no.	Mean value of percent growth inhibition ± SD					
	F. moniliformis	*A. specie*	*A. niger*	*F. solani*	*M. specie*	*A. fumigatus*
1	70.73 ± 1.88	0 ± 0.00	29.04 ± 0.25	14.35 ± 0.49	0 ± 0.00	1.93 ± 2.12
2	60.23 ± 1.27	54.98 ± 0.16	77.56 ± 1.41	27.87 ± 4.94	30.85 ± 2.12	20.95 ± 0.14
3	55.46 ± 1.41	65.33 ± 0.75	73.69 ± 0.30	36.57 ± 0.46	37.68 ± 0.70	27.46 ± 0.17
4	100 ± 0.00	87.69 ± 0.16	90.95 ± 0.46	45.64 ± 0.70	73.33 ± 3.53	0.72 ± 0.17
5	80.24 ± 0.49	05.02 ± 1.41	42.01 ± 0.28	37.43 ± 1.41	0 ± 0.00	5.31 ± 1.41
6	74.79 ± 0.70	87.48 ± 1.88	86.17 ± 0.46	32.82±3.53	66.6 ± 5.65	66.6 ± 0.25
HL	21.95 ± 2.12	48.71 ± 5.65	57.65 ± 2.82	0 ± 0.00	0 ± 0.00	19.56 ± 0.37
Terbinafine	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00

[a] *In vitro*, agar tube dilution method, concentration: 200 μg/mL of DMSO, percent inhibition (standard drug) = 100%, reference drug, Terbinafine.

Table 5
Cytotoxicity and antitumor activities of 6-nitropiperonylic acid and its organotin(IV) derivatives.[a,b]

Compound no.	LD_{50} (μg/mL)	Average number of tumors ± SE	% Inhibition of tumors
1	149.37	3.7 ± 0.36	61.1
2	–	1.3 ± 1.13	87.3
3	–	2.4 ± 0.61	74.7
4	0	0 ± 0.00	100.0
5	75.47	3.0 ± 0.51	51.6
6	0	0 ± 0.00	100.0
HL	230.78	9.3 ± 0.69	31.5

[a] *In vitro*, against brine-shrimps, reference drug MS-222 (Tricaine Methanesulfonate).
[b] Potato disc antitumor assay, concentration: 1000 ppm in DMSO, more than 20% tumor inhibition is significant, data represents mean value of 15 replicates, reference drug, Vincristine.

Potato Disc Antitumor assay was also performed for all these synthesized compounds by using Agrobacterium tumefaciens (At10) [21] (Table 5). All the compounds showed significant level of tumor inhibition in comparison to reference drug (Vincristine) with 100% tumor inhibition, as shown in Table 5. Activity of the synthesized organotin series was observed more than their parent acid. Furthermore, compounds **4** and **6** showed 100% tumor inhibition.

4. Conclusion

Six new organotin compounds of 6-nitropiperonylic acid were synthesized by reacting either sodium salt with organotin chlorides or ligand acid with organotin oxides and were characterized by FT-IR and ^{1}H, ^{13}C and ^{119}Sn multinuclear NMR. Single crystal X-ray analysis of compound **4** showed distannoxane with two types of tin atoms. Endocyclic Sn is six coordinated with skew trapezoidal bipyramidal geometry while exocyclic Sn is five coordinated with distorted trigonal bipyramidal geometry. All the synthesized organotin compounds revealed better *in vitro* biological activities than their parent acid when screened for antibacterial, antifungal, cytotoxicity and potato disc antitumor studies.

Acknowledgements

M. Hanif is indebted to Pakistan Atomic Energy Commission (PAEC) for granting study leave. M. Hanif, M. Hussain and M.S. Hussain highly acknowledge the financial grant from the Higher Education Commission, Pakistan.

Appendix A. Supplementary data

CCDC 727617 contains the supplementary crystallographic data for **4**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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