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Synthesis, Spectroscopic Investigation, Crystal Structure, and Biological Screening, Including Antitumor Activity, of Organotin(IV) Derivatives of Piperonylic Acid

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We prepared 8 new organotin compounds with the general formulae [R_3SnL], where R = Me (**1**), Bu (**2**), Ph (**3**), and Cy (**4**) and [R_2SnL_2], where R = Me (**5**), Et (**6**), Bu (**7**), Oct (**8**), and L = piperonylic acid, and characterized them by elemental analyses, IR spectra, and multinuclear NMR (^{1}H, ^{13}C, and ^{119}Sn). The structure of **5** was confirmed by single crystal X-ray crystallography. The geometry around the tin atom was compared in both solution and solid state. Crystals of **5** are monoclinic with the space group $P2_1/c$. The tin geometry is skew trapezoidal bipyramidal, with 2 longer and 2 shorter Sn-O bonds; thus, the ligand chelates the Sn center in an asymmetric way. The synthesized compounds were also screened for antibacterial, antifungal, brine-shrimp lethality, and potato disc antitumor activity.

Key Words: Organotin(IV) compounds, FT-IR, multinuclear NMR, X-ray structures, antibacterial, antifungal, brine-shrimp lethality, antitumor activities.

Introduction

Organotin(IV) compounds of the carboxylic acids are being extensively studied with special reference to their methods of synthesis, structural elucidation, and biological activity.[1−7] Generally, these compounds are well characterized by multinuclear NMR (^{1}H, ^{13}C, and ^{119}Sn), X-ray, and ^{119m}Sn Mossbauer spectroscopy.[8−10] In recent years, organotin(IV) carboxylates have attracted much attention due to their potential biocidal activity and cytotoxicity.[1,2,5,6,11] In fact, among the organometallic compounds, organotin carboxylates

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have received increased interest due to their activity against various types of cancer. Many of the di-*n*-butyltin(IV), tri-*n*-butyltin(IV), and triphenyltin(IV) complexes display interesting antitumor activity. Another aspect of major interest in organotin carboxylates is their structural diversity. Both diorganotin and triorganotin esters show rich and diverse structural chemistry, as citied in recent reviews.[5,8] Keeping in view the structural and biological diversity of organotin(IV) carboxylates and in connection with our interest in coordination chemistry of organotin compounds with different carboxylic acids[12−20], herein we present the synthesis, characterization, and in vitro biological activity of a carboxylic acid, piperonylic acid (Figure 1), and its organotin compounds.

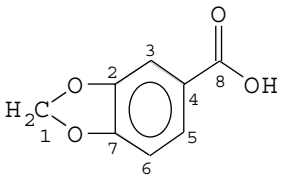

Figure 1. Numbering scheme and structure of the piperonylic acid (HL).

Experimental

Materials

All the organotin precursors and the ligand piperonylic acid were procured from Aldrich or Fluka. All the solvents were dried as described in the literature.[21]

Instrumentation

Melting points were determined in capillary tubes using an MPD Mitamura Riken Kogyo (Japan) electrothermal melting point apparatus and were uncorrected. IR absorption spectra were recorded as KBr pellets or neat liquid on a Bio-Rad Excalibur FT-IR model FTS 3000 MX spectrometer (USA). ^{1}H-, ^{13}C-, and ^{119}Sn-NMR spectra were recorded on a Bruker AM 250 spectrometer.

Synthesis

General Procedure

Two different methods were employed for the synthesis of the organotin derivatives of the piperonylic acid. In method A, the organotin chloride was refluxed with the sodium salt of the acid in dry toluene for 5-6 h in a 1:2 (diorganotin dichloride) or 1:1 (triorganotin chloride) molar ratio. After reflux the insoluble material was filtered off and the solvent was evaporated under reduced pressure. The resultant solid masses were recrystallized from a chloroform and *n*-hexane mixture. In method B, appropriate amounts of R_2SnO and ligand were refluxed for 6 h in 1:2 molar ratios in dry toluene (100 mL) for azeotropic removal of water formed during the condensation reaction using a Dean-Stark apparatus. 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.

Compound (1) [Me_3SnL]

Yield 70%, mp 119-120 °C. Analysis Calculated for $C_{11}H_{14}O_4Sn$: C, 40.12; H, 4.26. Found: C, 40.23; H, 4.20. ^{1}H-NMR ($CDCl_3$, ppm, $^nJ(^1H, ^1H)$, $^nJ[^{119/117}Sn, ^1H]$ in Hz), 6.03 (s, 2H), 6.83 (d, 1H, 8.1), 7.68 (dd, 1H, 8.1,1.6), 7.51 (d, 1H, 1.6), {0.64 (s, 9H), $SnCH_3$, [58.3, 55.8]}. ^{13}C-NMR ($CDCl_3$, ppm, $^nJ[^{119/117}Sn, ^{13}C]$ in Hz), 101.5 (C-1), 150.9 (C-2), 107.7 (C-3), 125.6 (C-4), 125.9 (C-5), 110.1 (C-6), 147.4 (C-7), 171.0 (C-8), –2.6 [397.7, 381.1] ($SnCH_3$). ^{119}Sn-NMR ($CDCl_3$, ppm) 134.59. IR (KBr, cm^{-1}), 445 ν(Sn-O), 549 ν(Sn-C), 1624 νas(COO) 1439 νs(COO) $\Delta\nu$(COO) = 185.

Compound (2) [Bu_3SnL]

Yield 70%, viscous liquid. Analysis Calculated for $C_{20}H_{32}O_4Sn$: C, 52.74; H, 7.03. Found: C, 52.68; H, 7.18. ^{1}H-NMR ($CDCl_3$, ppm, $^nJ(^1H, ^1H)$), 6.01 (s, 2H), 6.81 (d, 1H, 7.9), 7.67 (d, 1H, 8.1, 1.6), 7.50 (d, 1H, 1.4), {1.65 (m), 1.35 (m), 1.25 (m), 0.92 (t) (27H), $SnCH_2CH_2CH_2CH_3$}. ^{13}C-NMR ($CDCl_3$, ppm), 101.9 (C-1), 151.2 (C-2), 108.3 (C-3), 126.0 (C-4), 126.7 (C-5), 110.6 (C-6), 147.8 (C-7), 171.3 (C-8), 17.9, 27.4, 28.2, 14.0 ($SnCH_2CH_2CH_2CH_3$). IR (KBr, cm^{-1}), 450 ν(Sn-O), 557 ν(Sn-C), 1626 νas(COO) 1442 νs(COO) $\Delta\nu$(COO) = 184.

Compound (3) [Ph_3SnL]

Yield 83%, mp 113-116 °C. Analysis Calculated for $C_{26}H_{20}O_4Sn$: C, 60.58; H, 3.88. Found: C, 60.46; H, 3.95. ^{1}H-NMR (DMSO-d_6, ppm, $^nJ(^1H, ^1H)$), 6.04 (s, 2H), 6.84 (d, 1H, 8.2), 7.81 (dd, 1H, 8.1, 2.0), 7.50 (d, 1H, 1.6), {7.55-7.75 (m, 15H, SnPh}. ^{13}C-NMR ($CDCl_3$, ppm), 106.7 (C-1), 152.2 (C-2), 112.8 (C-3), 133.5 (C-4), 134.0 (C-5), 114.2 (C-6), 148.5 (C-7), 173.6 (C-8), 129.6, 133.0, 133.8, 141.3 (SnPh). IR (KBr, cm^{-1}), 446 ν(Sn-O), 590 ν(Sn-C), 1624 νas(COO) 1447 νs(COO) $\Delta\nu$(COO) = 177.

Compound (4) [Cy_3SnL]

Yield 70%, viscous liquid. Analysis Calculated for $C_{26}H_{38}O_4Sn$: C, 58.54; H, 7.13. Found: C, 58.70; H, 6.99. ^{1}H-NMR ($CDCl_3$, ppm, $^nJ(^1H, ^1H)$), 6.04 (s, 2H), 6.84 (d, 1H, 8.1), 7.71 (dd, 1H, 8.1,1.6), 7.38 (d, 1H,1.7), {1.8–1.29 (m, 33H), SnCy}. ^{13}C-NMR ($CDCl_3$, ppm), 101.9 (C-1), 151.1 (C-2), 108.1 (C-3), 126.0 (C-4), 127.0 (C-5), 110.7 (C-6), 147.8 (C-7), 171.0 (C-8), 8.3, 29.4, 31.6, 27.3 (SnCy). IR (KBr, cm^{-1}), 452 ν(Sn-O), 554 ν(Sn-C), 1625 νas(COO), 1440 νs(COO), $\Delta\nu$(COO) = 185.

Compound (5) [Me_2SnL_2]

Yield 73%, mp 217-220 °C. Analysis Calculated for $C_{18}H_{16}O_8Sn$: C, 45.10; H, 3.34. Found: C, 44.98; H, 3.41. ^{1}H-NMR ($CDCl_3$, ppm, $^nJ(^1H, ^1H)$, $^nJ[^{119}Sn, ^1H]$ in Hz), 6.08 (s, 4H), 6.88 (d, 2H, 8.2), 7.44 (dd, 2H, 8.0,1.5), 7.42 (d, 2H, 1.5), {1.13 (s, 6H), $SnCH_3$, [82.0]}. ^{13}C-NMR ($CDCl_3$, ppm, $^nJ[^{119/117}Sn, ^{13}C]$ in Hz), 101.8 (C-1), 152.0 (C-2), 107.9 (C-3), 123.7 (C-4), 126.5 (C-5), 110.3 (C-6), 147.7 (C-7), 175.5 (C-8), 4.8 [654.3, 625.6] ($SnCH_3$). IR (KBr, cm^{-1}), 460 ν(Sn-O), 585 ν(Sn-C), 1630 νas(COO), 1449 νs(COO), $\Delta\nu$(COO) = 181.

Compound (6) [Et_2SnL_2]

Yield 85%, mp 177-179 °C. Analysis Calculated for $C_{20}H_{20}O_8Sn$: C, 47.34; H, 3.94. Found: C, 47.43; H, 3.99. ^{1}H-NMR ($CDCl_3$, ppm, $^nJ(^1H, ^1H)$, $^nJ[^{119}Sn, ^1H]$ in Hz), 6.07 (s, 4H), 6.88 (d, 2H, 8.2), 7.77 (dd, 2H, 8.1,1.5), 7.58 (d, 2H,1.6), {1.78 (q, 4H, [75], (8.1)), 1.35 (t, 6H, (8.0)) $SnCH_2CH_3$}. ^{13}C-NMR ($CDCl_3$, ppm, $^nJ[^{119/117}Sn, ^{13}C]$ in Hz), 101.8 (C-1), 151.9 (C-2), 107.9 (C-3), 123.9 (C-4), 126.4 (C-5), 110.3 (C-6), 147.6 (C-7), 175.5 (C-8), 17.7 [611.3, 584.3], 9.0 [43] ($SnCH_2CH_3$). ^{119}Sn-NMR ($CDCl_3$, ppm) -159.23. IR (KBr, cm^{-1}), 456 ν(Sn-O), 548 ν(Sn-C), 1626 νas(COO) 1439 νs(COO) $\Delta\nu$(COO) = 187.

Compound (7) [Bu_2SnL_2]

Yield 78%, mp 117-119 °C. Analysis Calculated for $C_{24}H_{28}O_8Sn$: C, 51.15; H, 4.97. Found: C, 51.32; H, 4.80. ^{1}H-NMR ($CDCl_3$, ppm, $^nJ(^1H, ^1H)$ 6.08 (s, 4H), 6.89 (d, 2H, 8.1), 7.78 (dd, 2H, 8.2, 1.6), 7.59 (d, 2H,1.6), {1.82 (m), 1.47 (m), 1.38 (m), 0.93 (t) (18H), $SnCH_2CH_2CH_2CH_3$}. ^{13}C-NMR ($CDCl_3$, ppm) 102.2 (C-1), 152.3 (C-2), 108.3 (C-3), 124.4 (C-4), 126.8 (C-5), 110.7 (C-6), 148.0 (C-7), 175.6 (C-8), 25.8, 26.8, 27.1, 14.0 ($SnCH_2CH_2CH_2CH_3$). ^{119}Sn-NMR ($CDCl_3$, ppm)-154.51. IR (KBr, cm^{-1}), 458 ν(Sn-O), 588 ν(Sn-C), 1626 νas(COO) 1449 νs(COO) $\Delta\nu$(COO) = 177.

Compound (8) [Oct_2SnL_2]

Yield 78%, mp 104-106 °C. Analysis Calculated for $C_{32}H_{44}O_8Sn$: C, 56.80; H, 6.51. Found: C, 55.91; H, 6.04. ^{1}H-NMR ($CDCl_3$, ppm, $^nJ(^1H, ^1H)$ 6.06 (s, 4H), 6.88 (d, 2H, 8.1), 7.70 (dd, 2H, 8.2.1.1), 7.57 (d, 2H, 1.6), {1.80–1.22 (m), 0.85 (t) (34H), $SnCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$}. ^{13}C-NMR ($CDCl_3$, ppm) 102.2 (C-1), 152.2 (C-2), 108.3 (C-3), 124.4 (C-4), 126.8 (C-5), 110.7 (C-6), 148.0 (C-7), 175.5 (C-8), 25.0, 23.0, 33.6, 29.5, 29.4, 32.2, 26.4, 14.0 ($SnCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_3$). ^{119}Sn-NMR ($CDCl_3$, ppm) -153.29. IR (KBr, cm^{-1}), 458 ν(Sn-O), 559 ν(Sn-C), 1626 νas(COO), 1439 νs(COO), $\Delta\nu$(COO) = 187.

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 systems.[22,23] All data were collected with graphite-monochromated MoK_α radiation (λ = 0.71073 Å) at 173 K. Table 1 presents the crystallographic data for the compound **(5)**.

Biological activity

Antibacterial assay

All these synthesized compounds and their acids were tested against 6 bacterial strains; 3 gram-positive [*Bacillus subtilis* (ATCC 6633), *Micrococcus leuteus* (ATCC 10240), and *Staphylococcus aureus* (ATCC 6538)] and 3 gram-negative [*Escherichia coli* (ATCC 15224), *Enterobacter aerogenase* (ATCC 13048), and *Bordetella bronchiseptica* (ATCC 4617)]. The agar well-diffusion method was used for the determination of inhibition zones and minimum inhibitory concentration (MIC).[24] Briefly, 0.75 mL of the broth culture

containing ca. 10^6colony 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 diameter 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 a negative control and the standard antibacterial drugs roxithromycin (1 mg/mL) and cefixime (1 mg/mL) were used as positive controls. Triplicate plates of each bacterial strain were prepared. The plates were incubated aerobically at 37 °C for 24 h. Activity was determined by measuring the diameter of zone showing complete inhibition (mm) with the aid of a vernier caliper (precision: ± 0.1 mm). Growth inhibition was calculated with reference to the positive control. For individual compounds that showed inhibition > 10 mm, MIC values were determined by using the agar well-diffusion method.[24]

Table 1. Crystal data, data collection, and refinement details for compound **5.**

Empirical formula	$C_{18}H_{16}O_8Sn$
Formula weight	479
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	
a (Å)	7.8476(7)
b (Å)	10.7856(7)
c (Å)	21.4293(19)
α (°)	90
β (°)	99.725(11)
γ (°)	90
V (Å^3)	1787.7(3)
Z	4
D_c (g cm^{-3})	1.780
Crystal size (mm)	0.45 × 0.35 × 0.25
F(000)	952
Total reflections	8000
Independent reflections	3418
All indices (all data)	R_1 = 0.0234, wR_2 = 0.0497
Final R indices [I > 2σ(I)]	R_1 = 0.0195, wR_2 = 0.0487
Goodness of fit	1.029
θ Range for data collection (°)	2.12 to 25.94

Antifungal assay

Antifungal activity against 6 fungal strains [*Fusarium moniliformis, Alternaria* species*, Aspergillus niger, Fusarium solani, Mucor* species, and *Aspergillus fumigatus*] was determined with the agar tube dilution method.[24] Screw-caped test tubes containing Sabouraud dextrose agar (SDA) medium (4 mL) were autoclaved at 121 °C for 15 min. The tubes were allowed to cool to 50 °C and non-solidified SDA was loaded with 66.6 μL of compound pipetted from the stock solution (12 mg/mL in DMSO) to make 200 μL/mL final concentration. Tubes were then allowed to solidify in a slanting position at room temperature. Each tube was inoculated with a 4-mm diameter piece of inoculum from 7-day-old fungal culture. The media supplemented with DMSO and terbinafine (200 μL/mL) were used as negative and positive controls, respectively. The tubes were incubated at 28 °C for 7 days; then growth was determined by measuring linear growth

(mm) and growth inhibition was calculated with reference to the negative control.

Antitumor Activity

Antitumor potato disc assay[24] was also performed for all these synthesized compounds. Potato discs (0.5 cm thickness) were obtained from surface sterilized potatoes using a metallic cork borer and special cutter under complete aseptic conditions. These potato discs were then transferred to petri dishes, 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* (At 10, 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 dishes were incubated at 28 °C. After 21-day incubation, the number of tumors was counted with the aid of a dissecting microscope after staining with Lugol's solution.

Cytotoxicity

Cytotoxity was studied by the brine-shrimp lethality assay method.[24] Brine-shrimp (*Artemia salina*) eggs were hatched in artificial sea water (3.8 g sea salt/L) at room temperature (22-29 °C). After 2 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 the number of surviving shrimps was counted. Data were analyzed with a Finny computer program (probit analysis) to determine LD_{50} values. The results are summarized in Table 6.

Results and Discussion

The synthesis of organotin derivatives of piperonylic acid may be represented by the following equations:

$$R_3SnCl + NaL \longrightarrow R_3SnL + NaCl$$

R = Me (**1**), Bu (**2**), Ph (**3**), Cy (**4**)

$$R_2SnCl_2 + 2NaL \longrightarrow R_2SnL_2 + 2NaCl$$

R = Me (**5**), Et (**6**)

$$R_2SnO + 2LH \longrightarrow R_2SnL_2 + H_2O$$

R = Bu (**7**), Oct (**8**)

IR spectra

Vibrational data of the synthesized compounds are collected in the experimental part and the coordination mode of the ligand acid towards the di- and triorganotin(IV) moieties can be deduced by comparing the IR spectra of the free acid, its salt, and organotin compound. Diagnostically important IR bands are ν_{as}(COO), ν_s(COO), ν(Sn–C), and ν(Sn–O). The magnitude of $\Delta\nu$(COO) is in the range of 177-187 cm^{-1},indicating a bidentate nature of the carboxylate towards the Sn atom.[25] Thus, according to earlier reports featuring the same results and crystallographic data, it is most likely that in diorganotin compounds (**5-8**) the tin atom

approaches 6-coordination based on the skew trapezoidal planar geometry[25] and the carboxylate group acts as an asymmetric bidentate ligand. Fortunately, the X-ray crystal structure of compound **5** supports the IR data for the diorganotin compounds. The X-ray crystal structure of compound **5** will be described in the following section. The bidentate nature of the carboxylate ligand also suggests 5-coordinated tin atoms in the triorganotin compounds **(1-4),** with trigonal bipyramidal geometry. In accordance with earlier reports, triorganotin carboxylates with bridging ligands lead to *trans*-R_3SnO_2 geometry for tin.[13]

X-Ray structure of 5

The molecular structure of compound **5** is depicted in Figure 2, while selected geometric parameters are given in Table 2. Compound **5** has adopted the skew trapezoidal bipyramidal structural motif about the Sn1 atom and reveals a monomeric molecule. The 4 oxygen atoms of chelating carboxylate ligands coordinate to the Sn1 atom, forming 2 shorter [Sn1-O1 = 2.125(14) and Sn1-O5 = 2.130(13) Å] and 2 longer [Sn1-O2 = 2.502(13) and Sn1-O6 = 2.526(13) Å] Sn-O bonds, which reflect that the carboxylate ligands chelate the Sn1 center in an asymmetric way. The 2 oxygen atoms lie in the equatorial plane, while C1 and C2 atoms are in axial positions. The 2 short Sn-O bonds are *cis* to one another, with a very acute angle [O1-Sn1-O5 = 81.72(5)°]. The longer Sn-O bonds [with O2-Sn1-O6 = 166.97(5) angle] lie nearly 13° short of being linear to each other. The longer Sn-O bonds are much longer than the sum of the covalent radii of the tin and oxygen (2.13 Å) as compared to the shorter Sn-O bonds, but significantly below the sum of the van der Waal's radii of these atoms (3.68 Å).[8] The C-Sn-C angle, C2-Sn1-C1 = 149.53(10)°, is distorted from a true *trans* position by nearly 31°, so as to better occupy the open space left by the skew trapezoidal arrangement of the equatorial ligands.[9] The anisobidentate mode of coordination of the carboxylate ligands are also accompanied by unequal C-O bonds, [C3-O2 1.240(3), C3-O1 1.301(2) , C11-O6 1.243(2), C11-O5 1.293(2) Å]. The Sn-C bonds [C1-Sn1 2.101(2) and C2-Sn1 2.100(2) Å] are similar to those found in earlier reports. These observations are in excellent agreement with the coordination geometries found for diorganotin dicarboxylates[8] in which the carboxylate ligands chelate the tin atom, forming asymmetric type Sn-O bonds in which the short Sn-O bonds range from 2.07 to 2.16 Å, while the long Sn-O bonds range from 2.45 to 2.65 Å.[8,9] The geometry about the tin atom is regarded as a skew trapezoidal bipyramid with the tin-bound organic groups being disposed in pseudo-axial positions over the weaker equatorial Sn-O interactions to give C-Sn-C angles in the range of 130-152°.[8] The structures exhibiting similar geometry to compound **5** include $(C_2H_5)_2Sn(O_2CCHCHC_4H_3S)_2$,[26] $(CH_3)_2Sn(O_2CPh)_2$,[27] $(CH_3)_2Sn(O_2CCH_3)_2$,[28] and $(C_2H_5)_2Sn(O_2CC_4H_3S)_2$.[29]

NMR spectra

The ^{1}H-NMR spectral data of the ligand show single resonance at 9.81 ppm, which is absent in the spectra of the complexes, indicating the replacement of the carboxylic acid proton by the organotin moiety. In addition, the resonance appearing at 6.13 ppm as a singlet is attributed to the O-CH_2-O protons and aromatic protons appearing in the expected region. In the complexes, a set of similar patterns of the signals has been observed. The methyl protons in trimethyltin (**1**) and dimethyltin (**5**) derivatives appear as sharp singlets with $^2J[^{119}Sn,^1H]$ coupling of 58.3 and 82 Hz, respectively. In the case of diethyltin **(6)**, the ethyl protons show a chemical shift at 1.78 and 1.35 ppm for the $SnCH_2CH_3$ fragment, respectively, with expected

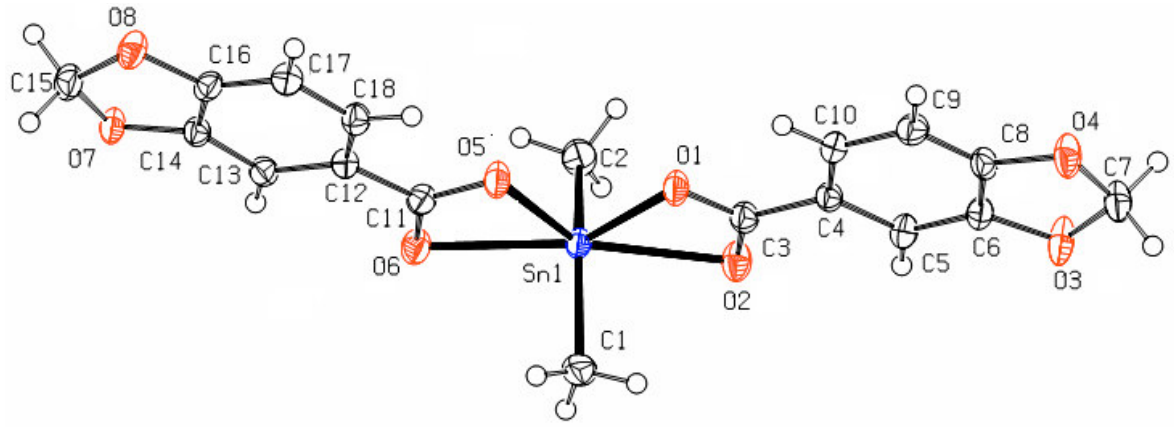

Figure 2. Molecular structure of compound **5** with atom-numbering scheme. Displacement ellipsoids are shown at 50% probability level.

multiplicity and $^{2}J[^{119}Sn,^{1}H]$ coupling of 75 Hz. The *n*-butyl protons in **2** and **7** show a complex pattern due to CH_2-CH_2-CH_2-skeleton in the range of 1.82-1.25 ppm and a clear triplet due to the terminal methyl groups at 0.92 and 0.93 ppm, respectively. As expected, the aromatic protons of **3** and the cyclohexyl protons of **4** show a complex pattern at 7.75-7.55 and 1.80-1.29 ppm, respectively. The methylene protons' $(CH_2)_7$ moiety of complex **8** exhibit a chemical shift in the range of 1.80-1.22 ppm and terminal methyl protons appear as a triplet at 0.85 ppm.

Table 2. Selected bond lengths and angles (Å, °) for compound **5.**

C1-Sn1	2.101(2)	C2-Sn1	2.100(2)
O1-Sn1	2.125(14)	O2-Sn1	2.502(13)
O5-Sn1	2.130(13)	O6-Sn1	2.526(13)
C3-O2	1.240(3)	C3-O1	1.301(2)
C11-O6	1.243(2)	C11-O5	1.293(2)
C2-Sn1-C1	149.53(10)	C2-Sn1-O1	100.70(7)
C1-Sn1-O1	100.14(7)	C2-Sn1-O5	102.54(7)
C1-Sn1-O5	102.25(8)	O1-Sn1-O5	81.72(5)
C2-Sn1-O2	86.61(7)	C1-Sn1-O2	86.84(7)
O1-Sn1-O2	56.06(5)	O5-Sn1-O2	137.78(5)
C2-Sn1-O6	90.07(7)	C1-Sn1-O6	89.68(7)
O1-Sn1-O6	136.96(5)	O5-Sn1-O6	55.24(5)
O2-Sn1-O6	166.97(5)		

The assignment of ^{13}C-NMR signals for –COO, O-CH_2-O, and phenyl carbon atoms of the ligand acid is straightforward and they are assigned by comparison to related organic analogues.[30] The complete assignments of the ^{13}C-NMR signals of the compounds are based on comparison with the ligand and related analogues as model compounds,[31,32] and in some cases with $^{n}J[^{119}Sn,^{13}C]$ coupling constants. The coupling constants $^{1}J[^{119}Sn,^{13}C]$ are important indicators for structural evaluation of organotin carboxylates. Holeček and coworkers[33,34] have shown that for 4-coordinated trialkyltin compounds the coupling constant, $^{1}J[^{119}Sn,^{13}C]$, occurs in the range of 325-400 Hz, while 5-coordinated tin compounds exhibit couplings in the range of 440-540 Hz.[35,36] We observed the $^{1}J[^{119}Sn,^{13}C]$ coupling satellite of the order of 397.7 Hz in

trimethyltin (**1**) characteristic of the tetrahedral compounds. The calculated θ (C-Sn-C) by the Lockhart and Holeček equations is 110.8°, which is close to the ideal tetrahedral angle.[37,38] Thus, the bidentate nature of the ligand acid resulting in solid state is therefore lost in solution to generate a monomeric 4-coordinated tetrahedral structure for triorganotin compounds. For other triorganotin compounds, we were not able to observe the couplings, but we assumed a monomeric 4-coordinated tetrahedral structure for compounds **2**-**4**. The magnitudes of $^{1}J[^{119}\mathrm{Sn},^{13}\mathrm{C}]$ coupling satellites in diorganotin compounds were observed for compounds **5** and **6,** which suggests a coordination number more than 4 in comparison with earlier reports.[39]

It is reported that in the organotin carboxylates, 4-, 5- and 6-coordinate tin have $\delta(^{119}\mathrm{Sn})$ values ranging from +200 to –60 ppm, from –90 to –190 ppm, and from –200 to –400 ppm, respectively.[7] We recorded ^{119}Sn-NMR for some compounds and the values were 134.59 ppm for **1**, –159.23 ppm for **6**, –154.51 ppm for **7,** and –153.29 ppm for **8**. A single resonance at 134.59 ppm for the trimethyltin derivative is compatible with tetrahedral geometry around the tin atom in solution. This suggests that the bidentate nature of the carboxylate is lost in the solution and the ligand behaves as a monodentate. For diorganotin compounds, the ^{119}Sn-NMR values ranged from –153.29 to –159.23 ppm and fall in the category of penta-coordinated tin in solution.[14,31]

Biological activity

In vitro biocidal screening tests of the synthesized compounds and their acids were carried out for antibacterial, antifungal, and antitumor activity. Antibacterial activity was tested against 6 bacterial strains; 3 gram-positive [*Bacillus subtilis* (ATCC 6633), *Micrococcus leuteus* (ATCC 10240), and *Staphylococcus aureus* (ATCC 6538)] and 3 gram-negative [*Escherichia coli* (ATCC 15224), *Enterobacter aerogenase* (ATCC 13048), and *Bordetella bronchiseptica* (ATCC 4617)]. The agar well-diffusion method[24] was used in these assays and each experiment was performed in triplicate. Readings of the zone of inhibition represent the mean value of 3 readings with standard deviation (STDEV), which are shown in Table 3. Roxithromycin and cefixime were used as standard drugs in these assays. The data obtained show that most of the synthesized organotin series have some antibacterial activity. Compounds **6** and **7** have antibacterial activity against all 6 strains. Some compounds show even better activity than the reference drugs; however, compounds **1** and **8**, and their acids show no activity against any of the 6 strains. For individual compounds that showed inhibition $>$ 10 mm, MIC (minimum inhibitory concentration) values were determined using the agar well-diffusion method.[24] The tests were performed in triplicate and the averaged results are shown in Table 3.

All synthesized compounds were also subjected to antifungal activity testing against 6 fungal strains (*Fusarium moniliformis, Alternaria* species*, Aspergillus niger, Fusarium solani, Mucor* species, and *Aspergillus fumigatus*) using the agar tube dilution method.[24] The results are presented in Table 4. Turbinafine was used as the standard drug in this assay. All the synthesized organotin compounds, except **6**, showed more activity than their acids. In some cases, the activity of the synthesized compounds was equal to the reference drug. Compounds **1**-**4** had significant activity against most of the fungal strains tested.

Table 3. Antibacterial activity of the synthesized compounds.[a−d]

Compound no.	Zone of inhibition (mm)+STDEV					
	S. aureus	*B. subtillus*	*M. luteus*	*Ent. aerog*	*E. coli*	*Bor. bron.*
1	-	-	-	-	-	-
2	5.4 ± 0.20	-	-	-	2.33 ± 0.05	-
3	16.85 ± 0.24	1.8 ± 0.0	-	-	16.45 ± 0.76	-
4	3.0 3 ± 0.15	-	-	3.8 ± 0.1	-	-
5	-	-	-	3.66 ± 0.57	9.35 ± 0.89	-
6	12.25 ± 0.35	9.43 ± 1.72	8.26 ± 0.40	8.26 ± 0.46	9.05 ± 0.91	10.6 ± 0.21
7	5.75 ± 0.35	4.53 ± 0.68	3.46 ± 0.15	2.73 ± 1.06	6.25 ± 1.34	4.35 ± 0.07
8	-	-	-	-	-	-
Acid	-	-	-	-	-	-
Roxithromycin	17.4 ± 0.42	5.0 ± 0.80	5.0 ± 0.52	5.0 ± 0.35	5.0 ± 0.70	5.0 ± 0.07
Cefixime	23.85 ± 0.35	28.5 ± 0.26	28.5 ± 0.057	28.5 ± 0.49	28.5 ± 0.33	28.5 ± 0.3
DMSO	-	-	-	-	-	-

[a]-Show no activity.
[b]In vitro: Agar well-diffusion method; concentration: 1 mg/mL of DMSO.
[c]Reference drug: roxithromycin and cefixime 1 mg/mL DMSO.
[d]Criteria for activity:

Zone diameter	Activity
3-6 mm	Non-significant
7-9 mm	Low
10-12 mm	Good
> 12 mm	Significant

Table 4. Antifungal activity of the synthesized compounds.[a−d]

Compound no.	Percent growth inhibition ± SE					
	F. monil.	*Alternaria* sp.	*A. niger*	*F. solani*	*Mucor* sp.	*A. fumigatus*
1	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
2	79 ± 1.50	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
3	82 ± 0.28	100 ± 0.0	100 ± 0.0	88.3 ± 0.66	100 ± 0.0	95 ± 2.0
4	85.3 ± 0.33	86 ± 2.08	82 ± 1.15	86 ± 1.73	39 ± 1.50	73 ± 1.73
5	03 ± 0.57	21 ± 1.0	38 ± 1.15	27 ± 1.15	-	30 ± 0.57
6	-	-	-	-	-	-
7	50 ± 2.64	45 ± 2.08	60.6 ± 1.33	-	10 ± 1.0	45.3 ± 1.20
8	24.6 ± 0.33	02 ± 0.57	03 ± 1.0	70 ± 1.0	10 ± 1.50	57 ± 0.66
Acid	-	20.3 ± 1.45	04 ± 0.0	-	-	-
Turbinafine	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
Negative Control	-	-	-	-	-	-

[a]-Show no activity.
[b]In vitro agar tube dilution method, concentration: 200 μg/mL of DMSO.
[c]Percent growth inhibition of standard drug: 100%.
[d]Criteria for activity:

% inhibition	Activity
30-40	Low
50-60	Moderate
60-70	Good
> 70	Significant

Antitumor potato disc assay[24] was also performed for all the synthesized compounds using *Agrobacterium tumefaciens* (At 10). All the compounds showed a significant level of tumor inhibition, as shown in Table 5. Activity of the synthesized organotin series was observed more than their acids. Furthermore, compounds **2** and **3** showed 100% tumor inhibition.

Table 5. Antitumor activity of the synthesized organotin compounds.[a−c]

Compound no.	Average number of tumors ± SE	% inhibition of tumors
1	0.3 ± 0.15	96.84
2	0.0 ± 0.0	100
3	0.0 ± 0.0	100
4	0.1 ± 0.1	98.94
5	2.6 ± 0.49	72.63
6	5.7 ± 0.98	40.00
7	1.4 ± 0.49	85.26
8	2.4 ± 0.58	74.73
Acid	6.4 ± 1.04	32.63
Negative Control	9.5 ± 1.15	-

[a]Potato disc antitumor assay, concentration: 1000 ppm in DMSO.
[b]More than 20% tumor inhibition is significant.
[c]% inhibition of tumors = 100 - ns/nc × 100. (ns = number of tumor for sample and nc = number of tumors for control) Data represents mean value of 15 replicates.

Cytotoxicity was studied by the brine-shrimp assay method[24] and the results are summarized in Table 6. The LD_{50}data show that all the compounds, even the ligand acid, are toxic with LD_{50} values in the range of 0.0634-403.019 μg/mL.

Table 6. Cytotoxicity data of the synthesized organotin compounds.[a,b]

Compound no.	LD_{50}
1	0.0634
2	1.44
3	14.04
4	3.67
5	83.87
6	403.019
7	117.73
8	13.10
Acid	0.1149

[a]Against brine-shrimp (in vitro).
[b]Data are based on mean value of 3 replicates.

Conclusion

Eight new organotin complexes of piperonylic acid were synthesized by reacting the sodium salt/acid with the corresponding organotin and were characterized by different spectroscopic methods. Single crystal X-ray analysis of compound **5** showed skew trapezoidal bipyramidal geometry around the tin atom, indicating the unsymmetrical nature of the ligand coordination towards the tin atom. Some of the synthesized compounds

revealed better biological activity when screened for antibacterial, antifungal, cytotoxicity, and potato disc antitumor studies.

Supplementary material

Crystallographic data for the structural analysis for the complex are deposited at the Cambridge Crystallographic Data Centre, CCDC No. 623839. Copies of this information may be obtained on request from the Director, CCDC, 12 Union Road, Cambridge, CBZ 1EZ, UK (Fax: +44-1223-336033; email: deposit@ccdc.cam.ac.uk or www:http://www.ccdc.cam.ac.uk).

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