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Full Paper

Synthesis, Chemical Characterization and Biological Screening for Cytotoxicity and Antitumor Activity of Organotin (IV) Derivatives of 3,4-Methylenedioxy 6-nitrophenylpropenoic Acid

Muhammad Sheeraz Ahmad [1], Mukhtiar Hussain [2], Muhammad Hanif [2], Saqib Ali [2] and Bushra Mirza [1,*]

[1] Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad Pakistan; E-mail: sheeraz_qau@yahoo.com (Muhammad Sheeraz Ahmad)
[2] Department of Chemistry, Quaid-i-Azam University, Islamabad-45320, Pakistan

* Author to whom correspondence should be addressed; E-mail: bushra_dr@yahoo.com

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Abstract: A series of mono-, di- and triorganotin compounds with general formulae [$RSnL_2Cl$], R = Bu (compound **3**), [R_2SnL_2], where R = Me, Et, Bu, Oct (compounds **1**, **2**, **4** and **6**) and [R_3SnL], where R = Bu, Cy and Ph (compounds **5**, **7** and **8**) and where **L** = 3,4-methylenedioxy-6-nitrophenylpropenoic acid have been prepared and characterized by elemental analysis, multinuclear (^{1}H-, ^{13}C- and ^{119}Sn-) NMR and mass spectrometry. The ligand and its respective organotin complexes were screened for cytotoxicity using the brine shrimp lethality assay and for antitumor activity using the crown gall tumor inhibition (potato disc) assay. The bioassay results support the conclusion that the biological activities of these synthetic compounds are in the following order: [$RSnL_2Cl$] < [R_2SnL_2] < [R_3SnL].

Keywords: Antitumor; cytotoxicity; mass spectrometry; multinuclear NMR; organotin.

Introduction

Organotin (IV) complexes are extensively used as catalysts, stabilizers, biocides, antifouling agents and as wood preservatives [1-5]. Organotin (IV) derivatives of carboxylic acids are of special interest

with regards to their methods of synthesis, structural elucidation and biological activity [6-9]. Generally these compounds can be well characterized by multinuclear NMR (^{1}H-, ^{13}C- and ^{119}Sn-) spectroscopy [10, 12]. In recent years organotin (IV) carboxylates have attracted much attention owing to their potential biocidal activity and cytotoxicity [5, 12]. In addition, among organometallic compounds there has been increased interest in organotin carboxylates due to their activity against various types of cancer cells as many of the known di- and triorganotin (IV) carboxylates display interesting antitumor activities [12-15].

Their structural diversity was also of interest as both diorganotin and triorganotin esters show rich and diverse structural chemistry, as cited in a recent review [8]. Keeping in mind this structural and biological diversity of organotin (IV) carboxylates, we have now synthesized some organotin (IV) carboxylates of 3,4-methylenedioxy-6-nitrophenylpropenoic acid (**L**). The structures of these compounds have been evaluated by various analytical techniques such as elemental analysis, multinuclear NMR (^{1}H-, ^{13}C- and ^{119}Sn-) and mass spectrometry. The ligand **L** and its organotin (IV) derivatives have been screened for cytotoxicity using the brine shrimp lethality assay and for antitumor activity with the crown gall tumor inhibition assay (potato disc assay).

Results and Discussion

Synthesis

Mono-, di- and triorganotin complexes were synthesized by refluxing stoichiometric amounts of the ligand acid, R'COOH, with the corresponding oxides/hydroxides (Procedure I) or its sodium salt with the corresponding organotin chlorides in toluene (Procedure II), as summarized in equations I-V.

$$RSn(OH)_2Cl + 2HL \longrightarrow RSn(Cl)L_2 + 2H_2O \quad \text{(I)}$$

R= n-Bu (**3**)

$$R_2SnO + 2HL \longrightarrow R_2SnL_2 + H_2O \quad \text{(II)}$$

R = *n*-Bu (**4**), *n*-Oct (**6**)

$$R_2SnCl_2 + 2NaL \longrightarrow R_2SnL_2 + 2NaCl \quad \text{(III)}$$

R = Me (**1**), Et (**2**)

$$R_3SnCl + NaL \longrightarrow R_3SnL + NaCl \quad \text{(IV)}$$

R = *n*-Bu (**5**)

$$R_3Sn(OH)+HL \longrightarrow R_3SnL+H_2O \quad \text{(V)}$$

R= Cy (**7**), Ph (**8**)

All the newly synthesized compounds are air stable crystalline solids, soluble in common organic solvents, and their physical data is summarized in Table 1. The structures of these compounds and coordination behavior of metal have been addressed by different analytical techniques such as elemental analysis, multinuclear NMR (^{1}H, ^{13}C and ^{119}Sn) and mass spectrometry.

Table 1. Physical data of 3,4-methylenedioxy-6-nitrophenylpropenoic acid (**L**) and its organotin (IV) derivatives.

Compound No.	Molecular Formula	Molecular Weight	M.P. (˚C)	Yield (%)	Elemental Analysis % Calculated (Found)		
					C	H	N
1	$C_{22}H_{18}O_{12}N_2Sn$	622	237-240	86	42.51 (42.48)	2.90 (2.96)	4.51 (4.58)
2	$C_{24}H_{22}O_{12}N_2Sn$	650	176-178	67	44.35 (44.40)	3.46 (3.43)	4.31 (4.42)
3	$C_{24}H_{26}O_{12}N_2SNCl$	689.5	142-145	70	42.13 (42.24)	3.07 (3.15)	4.10 (4.04)
4	$C_{28}H_{30}O_{12}N_2Sn$	706	196-199	73	47.66 (47.74)	4.26 (4.16)	3.97 (4.01)
5	$C_{22}H_{33}O_6NSn$	527	143-146	89	50.19 (50.23)	6.27 (6.30)	2.66 (2.70)
6	$C_{36}H_{46}O_{12}N_2Sn$	818	151-153	75	52.88 (52.96)	5.63 (5.60)	3.43 (3.50)
7	$C_{28}H_{39}O_6NSn$	605	99-101	83	35.63 (35.69)	6.46 (6.50)	2.32 (2.36)
8	$C_{28}H_{21}O_6NSn$	587	207-210	80	57.34 (54.43)	3.58 (3.60)	2.39 (2.45)
Ligand	$C_{10}H_7O_6N$	237	278	80	50.63 (50.45)	2.95 (2.80)	5.90 (5.87)

^{1}H-NMR Spectroscopy

^{1}H-NMR spectra for the synthesized compounds and free acid have been recorded in $CDCl_3$ and DMSO solutions. They are reported in Table 2. ^{1}H resonance signals of the protons attached to the phenyl moieties of the ligand have been assigned by their distinct multiplicities, *J*-values and comparison with the results obtained from the incremental method [9]. The methyl protons of dimethyltin derivatives appear as sharp singlets at 0.89 ppm, with well-defined satellites; coupling constants are included in Table 2. The α-CH_2 protons of diethyltin (IV) compounds resonate as a quartet at 1.52 ppm and have well-resolved coupling constants, while β-CH_3 protons resonate as a triplet at 1.34 ppm with $^3J[^1H,^1H]$ = 8.2 Hz. The phenyl moieties of the triphenyl- and diphenyltin (IV) derivatives mostly show a complex pattern and were assigned according to the literature [10]. Despite the complex pattern of the ^{1}H-NMR spectra of di- and tri-*n*-butyltin (IV) derivatives, assignments were possible: a clear triplet due to the terminal methyl group appears in the 0.91-0.92 ppm range [11,12a]. The α-CH_2 protons of the di-*n*-butyltin compound **4** appear as a triplet around 1.72 ppm, with $^3J[^1H,^1H]$ = 8.0 Hz [12b]. The β-CH_2 and γ-CH_2 signals appear as multiplets and δ-CH_3 as a triplet at 0.91 with $^3J[^1H,^1H]$ = 10.0 Hz. In the tri-*n*-butyltin (IV) complex, δ-CH_3 resonates as a triplet at 0.92 ppm with $^3J[^1H,^1H]$ = 8.5 Hz, and γ-CH_2 and β-CH_2 protons appear as multiplets at 1.33-1.37 ppm and

1.61-1.65 ppm, respectively. The α-CH_2 appears as a triplet at 1.60 ppm. The methylene protons (CH_2) of the *n*-octyltin (IV) moiety exhibit a somewhat different behavior, compared with the *n*-butyl groups of the respective complexes. The α-CH_2, β-CH_2 protons give multiplet signals at 1.49-1.53 ppm, respectively, which are consistent with the values calculated by the incremental method [13]. The γ-CH_2 and γ′-CH_2 do not appear in the region. However, the δ′-CH_3 protons resonate as a triplet in the range of 0.65 ppm with $^3J[^1H,^1H]$ = 7.0 Hz. The protons of the methylenedioxy moiety appear as singlets at 6.00-6.29 ppm and were seen in almost all the compounds. The $^2J[^{119}Sn\text{-}^1H]$ coupling constants and the C-Sn-C angles (Table 3) calculated from $^2J[^{119}Sn\text{-}^1H]$ values demonstrate that in the diorganotin (IV) derivatives, tin shows a coordination number greater that four, probably five or six in non coordinating solvents, while the triorganotin (IV) derivatives show distorted tetrahedral geometry [13, 14].

Table 2. ^{1}H-NMR data of 3,4-methylenedioxy-6-nitrophenylpropenoic acid and its organotin (IV) derivatives [a, b]

^{1}H No	Compound								
	Ligand	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2	7.09 (s)	7.12 (s)	7.00 (s)	7.10 (s)	7.09 (s)	7.01 (s)	7.02 (s)	7.03 (s)	6.96 (s)
5	7.52 (s)	7.58(s)	7.54 (s)	7.58 (s)	7.52 (s)	7.51 (s)	7.33 (s)	7.51(s)	7.78 (s)
7	6.29 (s)	6.24 (s)	6.12 (s)	6.00 (s)	6.11 (s)	6.14 (s)	5.98 (s)	6.14 (s)	6.11 (s)
8	7.82 (d,15.9)	7.96 (d,15.0)	8.17 (d,16.5)	7.95 (d,15.0)	7.99 (d, 15.0)	8.00 (d,15.0)	7.82 (d,16.0)	8.02 (d, 15.5)	7.82 (d,16.0)
9	6.50 (d,15.6)	6.47 (d,15.0)	6.33 (d,15.0)	6.47 (d,15.0)	6.30 (d, 15.0)	6.31 (d,15.0)	6.17 (d,15.5)	6.31 (d, 15.5)	6.30 (d,15.0)
α	–	0.89 (s)	1.52 (q, 10.0)	–	1.72 (t, 8.0)	1.6	1.49-1.53 (m)	–	–
β	–	–	1.34 (t, 8.2)	–	1.34-1.37 (m)	1.61-1.65 (m)	1.49-1.53 (m)	1.26-1.76 (m)	7.35-7.49 (m)
γ	–	–	–	–	1.34-1.37 (m)	1.33-1.37 (m)	–	1.26-1.76 (m)	7.35-7.49 (m)
δ	–	–	–	–	0.91 (t, 10.0)	0.92 (t, 8.5)	–	1.26-1.76 (m)	7.35-7.49 (m)
γ-γ′	–	–	–	–	–	–	1.15 (bs)	–	–
δ′	–	–	–	–	–	–	0.65 (t, 7.0)	–	–

[a] Chemical shifts (δ) in ppm. $^2J[^{119}Sn, ^1H]$; $^n[^1H, ^1H]$ in Hz where n= 3,4 are listed in square brackets and parenthesis, respectively. Multiplicity is given s=singlet, bs = broad signal, d = doublet, dd=doublet of doublet, q = quartet, m = multiplet; [b] Numbering in accordance with Figures 1- 4.

Figure 1. ^{1}H- and ^{13}C-NMR numbering scheme of the ligand **L.**

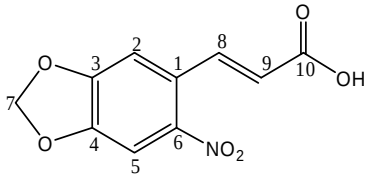


Table 3. (C–Sn–C) Angles (^{o}) Based on NMR Parameters of organotin (IV) derivatives of 3,4-methylenedioxy-6-nitrophenylpropenoic acid

Compound No.	**$^{1}J[^{119}Sn, ^{13}C]$ (Hz)**	**$^{2}J[^{119}Sn, ^{1}H]$ (Hz)**	**Angle (^{o})**	
			^{1}J	**^{2}J**
1	-	78	-	128.4
2	-	72	-	121.8
3	-	-	-	-
4	-	-	-	-
5	360	74	108.33	123.9
6	-	-	-	-
7	-	-	-	-
8	-	-	-	-

^{13}C-NMR Spectroscopy

^{13}C-NMR data recorded in $CDCl_3$ and DMSO solutions of the ligand and its respective mono-, di- and triorganotin (IV) derivatives are given in Table 4. The ^{13}C-NMR spectral data for the R groups attached to the tin atom where R = Me, Et, *n*-Bu, *n*-Oct, Ph and Cy were assigned by comparison with related analogues as model compounds, combined with the $^{n}J[^{119}Sn,^{13}C]$ coupling constants [15, 18]. The assignment of the ^{13}C- resonance associated with the carboxylate ligands is based: (I) on comparison with the results obtained from incremental methods [14], and (II) on comparison with the resonance values available in the literature [19]. The positions of the phenyl and olefinic carbon signals undergo minor variation in the complexes, compared to those observed in the free acid and its sodium salt. The carboxylate carbon shifts to a lower field region almost in all the complexes, indicating participation of the carboxyl group (COO) in coordination to tin (IV) [20].

Table 4. ^{13}C- and ^{119}Sn -NMR data[a,b] of 3,4-methylenedioxy-6-nitrophenylpropenoic acid and its organotin (IV) derivatives. [a, b]

^{13}C No	Ligand	1	2	3	4	5	6	7	8
1	125.78	126.36	126.96	127.11	127.86	127.93	127.25	128.07	127.53
2	107.19	105.93	107.64	107.65	107.46	107.37	107.68	107.39	107.40
3	151.73	153.10	152.05	152.62	151.90	151.90	153.09	151.87	151.94
4	148.73	150.18	149.24	148.78	148.65	148..63	150..29	148.55	148.87
5	106.90	104.86	105.18	105.01	105.60	105.56	105.75	105.56	105.63
6	138.87	141.35	142.67	143.81	138.96	139.03	141.52	138.78	140.88
7	103.74	104.30	103.06	103.45	103.26	103.25	104.68	103.20	103.20
8	142.98	141.25	143.92	143.81	143.13	143.06	144.34	143.07	143.10
9	122.67	123.10	120.78	124.54	125.82	124.54	122.49	124.85	122.77
10	167.03	187.87	175.39	186.84	171.62	170.90	174.75	170.70	171.96
α	-	4.34	19.17	22.70	22.70	15.24 [340, 360]	25.43	14.11	130.22
β	-	-	14.11	25.57	26.37	26.79	23.27	29.37	137.13
γ	-	-	-	29.19	26.83	27.04	33.56	31.95	128.97
δ	-	-	-	14.11	13.67	13.64	29.72	28.85	128.97
α'	-	-	-	-	-	-	31.34	-	-
β'	-	-	-	-	-	-	30.29	-	-
γ'	-	-	-	-	-	-	32.51	-	-
δ'	-	-	-	-	-	-	25.90	-	-
^{119}Sn	-	-112.63	-150.68	-150.03	-156.43	+116.10	-175.00	45.64	-112.45

[a] Chemical shifts (δ) in ppm. $^{n}J[^{117/119}Sn, ^{13}C]$; $^{n}J[^{119}Sn, ^{13}C]$; in Hz are listed in square brackets; [b] Numbering in accordance with Figures 1- 4.

Figure 2. Numbering scheme for groups attached to tin.

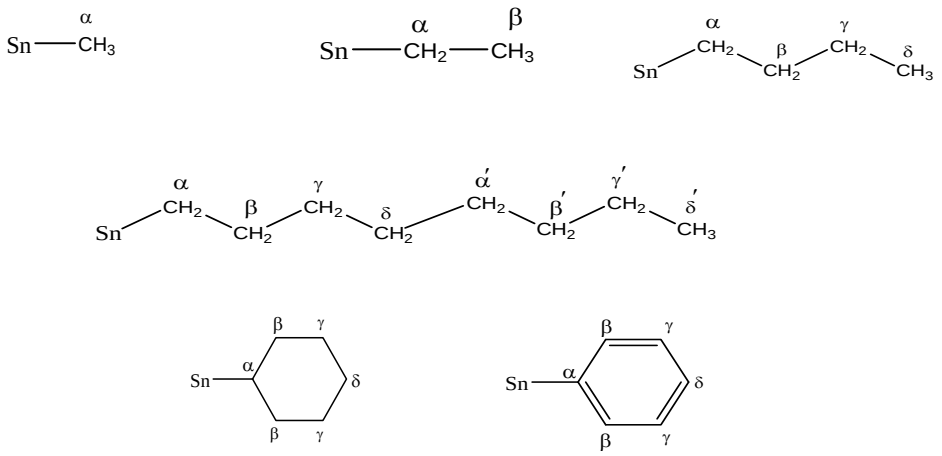
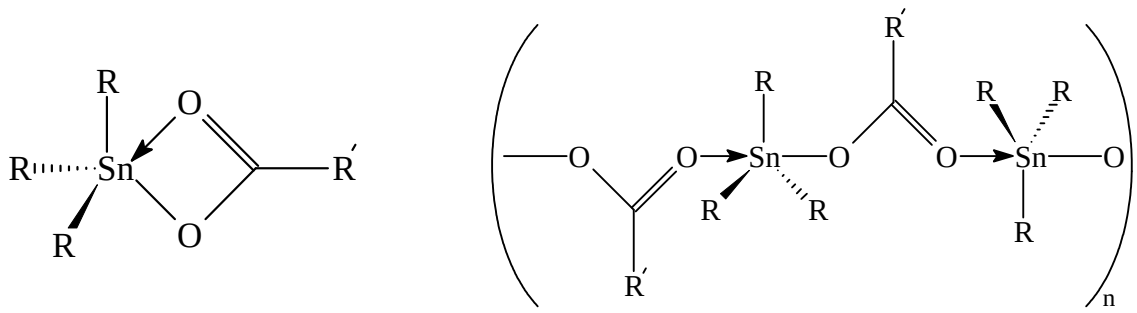


Figure 3. Structural diagrams of triorganotin (IV) Compounds.



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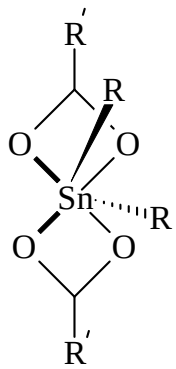


^{119}Sn-NMR Spectroscopy

The possibility of detecting the presence of coordinatively different organotin (IV) moieties was explored by acquisition of ^{119}Sn-NMR spectra for all the investigated compounds. The ^{119}Sn- chemical shifts usually cover a range, quoted relative to tetramethyltin, with increasing coordination number of tin producing a large upfield shift for δ(^{119}Sn). The ^{119}Sn-NMR spectra were recorded in $CDCl_3$ solutions (a non-coordinating solvent). It is reported [20, 21] that in alkyltin carboxylates, tetra-coordinated tin has δ(^{119}Sn) values ranging from about +200 to -60 ppm, and penta-coordinated tin from -200 to -400 ppm. A single resonance peak of ^{119}Sn chemical shift values obtained for the triorganotin (IV) derivatives lies in the range expected for a tetrahedral geometry. ^{119}Sn chemical shift values of diorganotin compounds were observed in the -112.63 to -175.0 ppm range. It appears from the shift values that a weakly hexacoordinated tin generates a deformed octahedron or a skewed trapezoidal bipyramid with four strong and two somewhat weaker bonds, as indicated by the upfield ^{119}Sn chemical shift values [14, 16, 20, 21]**.**

In order to gain further information about the possible coordination geometries in solution, close examination of the $^{1}J[^{119}Sn,^{13}C]$ and $^{2}J[^{119}Sn,^{1}H]$ coupling constants was undertaken as indicators of

structural details. As reported by Nadvornik *et al*. [13, 22a], ^{2}J [^{119}Sn, ^{1}H] in *n*-Bu_3Sn (IV) carboxylates changes very little with a change in coordination number, being the smallest among such couplings. In the case of diorganotin compounds **1** and **2**, the ^{2}J[^{119}Sn,^{1}H] values are 78 and 72 Hz, respectively, which indicate that tin displays hypervalent behavior (valence higher than four) in these compounds. .^{1}J[^{119}Sn,^{13}C] is, on the other hand, quite useful for making predictions about the geometry around the tin atom. In case of triorganotin (IV) carboxylates, ^{1}J[^{119}Sn,^{13}C] values ranging between 327 and 387 Hz for tetra-coordinated compounds and between 442 and 509 Hz for penta-coordinated ones were observed [22b]. For the tri-*n*-butyltin (IV) derivative the ^{1}J[^{119}Sn,^{13}C] values were 360 Hz and based on the Holeček and Lycka equation [20, 21], corresponds to a quasi-tetrahedral geometry in $CDCl_3$ solution. The geometric data calculated, as just described, are consistent with tetrahedral geometries for the triorganotin (IV) species [20], i.e. monomers in solution. Unfortunately, ^{1}J[^{119}Sn,^{13}C] values are not observed in any compound, but from the ^{119}Sn values and ^{2}J[^{119}Sn,^{1}H], it can be predicted that diorganotin (IV) species, possess skew-trapezoidal bipyramidal geometries with the lower apparent coordination number arising from the asymmetric coordination mode of the carboxylate ligand [23, 24].

Mass Spectrometry

The mass spectra of all the synthesized compounds **1-8** were determined using the electrospray ionization (ESI) technique. The molecular ion $[M]^+$ peak is not usually observed in case of organometallic compounds while using the conventional electron ionization (EI) or chemical ionization (CI) mass spectral techniques. However in case of ESI, not only the molecular ion peak $[M]^+$, but also peaks due to aggregation of fragments with $[M]^+$ or $[M+H]^+$ are encountered [25]. These fragment aggregates have molecular weights which is greater than $[M]^+$ (Table 5).

The 1st order +ve ESI-MS mode is more informative than the –ve mode because it can also provide information about the polymeric species and hence it is the more preferred for organotin species [26]. The neutral loses observed in ESI-MS are attributed to loss of CO_2, methane, ethane, butane and butene. The presence of more than one tin isotope in a molecule causes a wide distribution of the total signal among the many fragment aggregates, which decreases the relative intensity of the centroid peak. The presence or absence of a tin atom in individual ion can easily be recognized on the basis of the 10 characteristic natural tin isotopes, with the most abundant being the ^{120}Sn isotope.

The base peak in these compounds whether di- or triorganotin (IV) compounds derives from the different fragmentation patterns. All the investigated compounds were found to possess a base peak resulting from the loss of the ligand acid (**HL**), the R group or the ligand moiety itself.

Table 5. Mass fragmentation patterns of organotin (IV) derivatives of 3,4-methylenedioxy-6-nitrophenylpropenoic acid.

Fragment, m/z (%)							
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
$[C_{23}H_{20}N_2O_{12}Sn]^+$ 636 (8.5)	$[C_{22}H_{16}N_2O_{12}Sn]^+$ 620 (3.5)	$[C_{24}H_{19}ClN_2O_{12}Sn]^+$ 682.5 (2.5)	$[C_{32}H_{37}N_2O_{12}Sn]^+$ 761(16.5)	$[C_{30}H_{50}NO_6Sn]^+$ 640(7.3)	$[C_{40}H_{54}N_2O_{12}Sn]^+$ 874 (9.5)	$[C_{28}H_{39}NO_6Sn]^+$ 605 (2.5)	$[C_{22}H_{15}NO_6Sn]^+$ 509(14.9)
$[C_{22}H_{16}N_2O_{12}Sn]^+$ 620 (17.3)	$[C_{15}H_{12}NO_8Sn]^+$ 454 (41.6)	$[C_{20}H_{10}ClN_2O_{12}Sn]^+$ 625.5 (11.3)	$[C_{27}H_{26}N_2O_{12}Sn]^+$ 690 (7.6)	$[C_{22}H_{31}NO_6Sn]^+$ 525 (6.4)	$[C_{34}H_{40}N_2O_{12}Sn]^+$ 788 (16.2)	$[C_{27}H_{36}NO_4Sn]^+$ 558 (7.3)	$[C_{21}H_{13}NO_4Sn]^+$ 463 (3.3)
$[C_{21}H_{15}N_2O_{10}Sn]^+$ 575 (3.4)	$[C_{10}H_{13}O_4Sn]^+$ 317 (17.1)	$[C_{14}H_{14}ClNO_6Sn]^+$ 447.5 (32.7)	$[C_{24}H_{19}N_2O_{12}Sn]^+$ 647 (19.9)	$[C_{18}H_{23}NO_6Sn]^+$ 469 (23.6)	$[C_{28}H_{28}N_2O_{10}Sn]^+$ 672 (26.3)	$[C_{22}H_{27}NO_6Sn]^+$ 521 (33.4)	$[C_{15}H_8NO_4Sn]^+$ 386 (27.1)
$[C_{19}H_9N_2O_{10}Sn]^+$ 545 (37.7)	$[Sn]^+$ 120 (3.7)	$[C_5H_{11}Sn]^+$ 191 (41.5)	$[C_8H_{17}N_2O_{12}Sn]^+$ 453 (25.2)	$[C_{14}H_{14}NO_6Sn]^+$ 412 (12.7)	$[C_{18}H_{22}NO_6Sn]^+$ 468 (8.7)	$[C_{16}H_{16}NO_6Sn]^+$ 438 (17.1)	$[C_9H_6O_2Sn]^+$ 266 (72.4)
$[C_{11}H_6NO_6Sn]^+$ 368 (60.9)	$[C_4H_9]^+$ 57 (100)	$[C_4H_8Sn]^+$ 176 (6.9)	$[C_4H_7Sn]^+$ 175 (79.7)	$[C_7H_{10}O_2Sn]^+$ 246 (40.7)	$[C_9H_{16}O_2Sn]^+$ 276 (100)	$[C_9H_{12}O_2Sn]^+$ 272 (63.4)	$[C_{11}H_6NO_6Sn]^+$ 368 (60.9)
$[C_4H_4O_2Sn]^+$ 204 (100)	-	$[C_3H_8Sn]^+$ 164 (18.8)	$[C_2H_3Sn]^+$ 145 (100)	$[C_4H_9]^+$ 57 (100)	$[C_4H_9]^+$ 57 (76.3)	$[C_4H_6O_2Sn]^+$ 206 (100)	$[C_6H_5]^+$ 77 (100)
-	-	$[C_4H_9]^+$ 57 (100)	-	-	-	-	-

Biological Activity

All the synthesized compounds and their acid ligand were screened for their antitumor activity and cytotoxicity using standard i*n vitro* biocidal screening tests.

Cytotoxicity

Bioactive compounds are often toxic to shrimp larvae. Hence, shrimp larvae have been extensively used as rapid and simple preliminary test for cytotoxicity [11a, b]. All the synthesized compounds and ligand were screened for cytotoxicity by brine-shrimp bioassay lethality method [11b] and the results are summarized in Table 6. The LD_{50} data shows that all the compounds even the ligand acid are toxic with LD_{50} values in the range 0.26 to 581.23 μg/mL for organotin derivatives and 975.24 μg/mL for the ligand **L** Tri-organotin derivatives are more toxic than di-organotin derivatives, mono-organotin

derivatives and the ligand acid [27]. Compound **3** (a monoorganotin derivative) has been found to be the least toxic and compound **5** (a triorganotin derivative) was the most toxic of all the synthesized organotin derivatives [4, 5].

Table 6. Cytotoxicity data of organotin (IV) derivatives of 3,4-methylenedioxy-6-nitrophenylpropenoic acid.

Compound No	LD_{50} µg/mL
1	-
2	130.98
3	581.23
4	33.39
5	0.26
6	191.64
7	0.40
8	0.56
Ligand	975.24

Against brine shrimps (*in vitro*); Data is based on mean value of 3 replicates of each for 1000, 100 and 10 µg/mL concentrations; - = activity not determined.

Antitumor Activity

For antitumor activity, the crown gall tumor inhibition assay (potato disc assay) [11b] was performed for all the synthesized compounds using *Agrobacterium tumefaciens* (strain At10). Crown gall is a neoplasmic disease of plants induced by *Agrobacterium tumefaciens* due to its Ti (tumor inducing) plasmid [11b]. Since the mechanism of tumor induction is similar to that seen in animals, this assay was used to evaluate the antitumor activity of these compounds. All the compounds showed significant levels of tumor inhibition, as shown in Table 7. Activity observed for synthesized organotin series was more than that of their parent acid, and triorganotin derivatives showed more tumor inhibition than mono- and diorganotin derivatives. Furthermore, compounds **2**, **5** and **8** showed 100% tumor inhibition. The bioassays results reflect the conclusion that the activity are in the following order: $[RSnL_2Cl] < [R_2SnL_2] < [R_3SnL]$. Further, it has been observed that the activities of these mono-, di and triorganotin (IV) complexes are comparable with those reported earlier [2, 4, 5, 27].

Table 7. Antitumor activities of organotin (IV) derivatives of 3,4-methylenedioxy-6-nitrophenylpropenoic acid [a, b, c, d].

Compound No.	Average number of tumors ± SE	% inhibition of tumors
1	2.8±0.89	62.66
2	0.0±0.0	100
3	3.9±1.02	48
4	1.0±0.36	86.66
5	0.0±0.0	100
6	5.3±0.84	29.33
7	4.8±1.13	36
8	0.0±0.0	100
Ligand	4.02±1.04	44
(-)ive control	7.5±1.13	-

[a]) Potato disc antitumor assay, concentration: 1000 μg/mL in DMSO; [b]) More than 20% tumor inhibition is deemed significant; [c]) Percent Inhibition of tumors = [100 ─ (Number of tumors for sample/Number of tumors for control)] × 100; [d]) Data represents mean value of 15 replicates.

Experimental

General

Melting points were determined in a capillary tube using a MPD Mitamura Riken Kogyo (Japan) electrothermal melting point apparatus. 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] [10]. 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, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet). Mass spectral data (ESI mode) were taken on a LCQ Finnigan MAT ion trap (USA). The m/z values were evaluated assuming that H = 1, C = 12, N = 14, O = 16, Cl = 35.5 and Sn = 120.

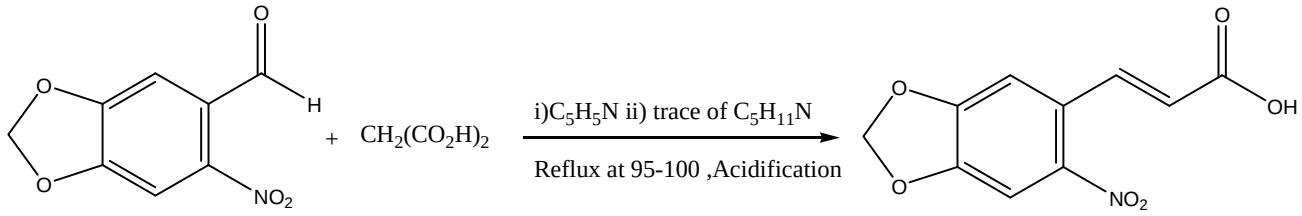
Synthesis

Different mono-, di- and triorganotin (IV) carboxylates were prepared by treating the corresponding ligand acid with organotin oxide/hydroxides or its sodium salt with organotin chlorides, adopting various standard procedures [28].

Synthesis of the Ligand **L**

The ligand acid 3,4-methylenedioxy 6-nitrophenylpropenoic acid was synthesized by dissolving 6-nitropiperonal (6.5 g, 0.033 mol) and malonic acid (7.5 g, 0.072 mol) in a mixture of pyridine (15 mL) and piperidine (0.25 mL) in a 100 mL round bottom flask and heating under reflux for 1 hour in an oil bath (Scheme 1). A rapid evolution of carbon dioxide took place. The reaction was completed by boiling the reaction mixture for 5 minutes, then it was cooled and poured into an excess of water containing enough hydrochloric acid to combine with the pyridine. The 3,4-methylendioxy-6-nitrophenylpropenoic acid was filtered off, washed with a little water and dried. The acid obtained was recrystallized from glacial acetic acid. The synthesized ligand acid is a crystalline solid, possessing a sharp melting point. And the yield is typically in the 67-86 % range.

Scheme 1.



Synthesis of Complexes

Procedure-I

The sodium salt RCOONa, was prepared by adding dropwise an equimolar amount of sodium hydrogen carbonate dissolved in distilled water to the ligand acid (RCOOH) dissolved in ethanol. The clear solution obtained was concentrated under reduced pressure. The dry sodium salt R'COONa (8 mmol), was mixed with diorganotin dichloride (4 mmol) or triorganotin chloride (8 mmol) in 2:1 and 1:1 ratios, respectively, in dry toluene contained in a 250 mL two necked round bottom flask. The mixture was refluxed for 9-10 hr. After refluxing a turbid solution was obtained, which was then left overnight at room temperature. The sodium chloride collected at bottom was filtered off and the solvent was evaporated by rotary evaporation. The resulting solid mass was recrystallized from chloroform and *n*-hexane mixture (4:1). Compounds **1**, **2** and **5** were prepared by this method in yields of 86, 67 and 89%, respectively.

Procedure-II

The ligand acid, R'COOH (12 mmol) and diorganotin oxide (6 mmol), or triorganotin hydroxide (12 mmol) in 2:1 and 1:1 ratios, respectively, were suspended in dry toluene (100 mL) in a single necked round bottom flask (250 mL), equipped with a Dean-Stark apparatus. Anhydrous conditions are required to avoid the oxidation of metal atom before the reaction. However, water is produced as a

secondary product and removed by the Dean-Stark apparatus. After the reaction is complete the products (tin carboxylates) are stable to moisture and oxygen. The mixture was refluxed for 8-10 hours and water formed during the condensation reaction was removed at regular intervals. It was then cooled to room temperature and solvent was removed by rotary evaporator. The solid obtained was recrystallized from chloroform containing a few drops of *n*-hexane. Compounds 3, 4, 6, 7, 8 were prepared by this method. Yield of these compounds were 70, 73, 75, 83, and 80%, respectively.

Biological Activity

Cytotoxicity

The cytotoxity was studied by the brine-shrimp lethality assay method [11b]. Brine-shrimp (*Artemia salina*) eggs (San Francisco Bay Brand, Inc, New York, CA94560, USA) were hatched in artificial sea water (40 g sea salts/L) at room temperature (22-29 ºC). Sea salts were obtained from from Instant Ocean, Inc, 8141, Tyler Boulevard, Mentor, OH44060, USA. After two days these shrimps were transferred to vials (10 shrimps per vial) containing artificial sea water (5 mL) with 1000, 100 and 10 μg/mL final concentrations of each compound taken from their stock solutions of 20 mg/mL in DMSO. After 24 hours number of surviving shrimps was counted. Data was analyzed with a finny computer programme (Probit analysis) to determine LD_{50} values.

Antitumor Activity

For antitumor activity crown gall tumor inhibition assay (Potato Disc Assay) [11b] was performed for all these synthesized compounds. Potato discs (0.5 cm thickness) were obtained from surface sterilized potatoes by using metallic cork borer (8 mm) and special cutter under complete aseptic conditions. These potato discs were then transferred to petri plates each containing 1.5 % agar (25 mL, 1.5 g agar/100 mL distilled water). Five potato discs were placed on each plate and three plates were used for each test sample along with same number of plates for control. Each compound (10 mg) was dissolved in DMSO (1 mL) in separate test tubes as a stock solution. Then stock solution of the test sample (0.5 mL, 10 mg/mL) was added to a broth culture of *Agrobacterium tumefaciens* (2 mL, At10, a 48 hours culture containing 5×10^9 cells/mL) and autoclaved distilled water (2.5 mL) to give 1000 μg/mL final concentration. These cultures (50 μL) were poured onto each potato disc. The petri plates were incubated at 28 ºC, the lids being taped down with Parafilm. After 21 days incubation, the number of tumors was counted with the aid of dissecting microscope after staining with Lugol's solution (5% I_2, 10% KI in distilled water).

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Sample Availability: Contact the authors.