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Spectroscopic Characterisation and Biological Applications of Organotin(IV) Derivatives of 3-(N-Naphthylaminocarbonyl)-2-propenoic Acid

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In an effort to develop new organotin materials for investigation and biocidal evaluation, a series of compounds with the general formula $R_{4-n}SnL_n$ (where R = CH_3, n-C_4H_9, C_6H_5, $C_6H_5CH_2$and L = 3-(N-naphthylaminocarbonyl)-2-propenoic acid) were synthesised, and characterised by elemental analysis, IR, multinuclear (^{1}H, ^{13}C and ^{119}Sn) NMR, ^{119m}Sn Mössbauer spectroscopy and mass spectrometry. The biological activity of these compounds against various bacteria and fungi was investigated. All of the compounds were active against the fungi tested with a few exceptions. These compounds also showed significant antibacterial activity. LD_{50} data show that the investigated compounds have significant toxicity.

Key Words: Organotin(IV) carboxylates, biological studies, spectroscopic studies.

Introduction

Extensive studies have been carried out for the structural characterisation of organotin carboxylates, both in solution and in solid states[1−4]. In addition to extensive biocidal applications, utilising the antitumour and anticancer activities of such compounds has made them significantly important[5]. The synthetic routes to organotin carboxylates have been studied in considerable detail. Depending upon the stoichiometry of the organotin precursors and carboxylic acid, several products such as monomers, dimers, tetramers, oligomer ladders and hexameric drums have been isolated[6]. Accordingly, and in view of our continuing interest in the synthesis, structural characterisation and biological applications of organotin derivatives of donor ligands containing chalcogens[7−15], we prepared a new series of organotin carboxylates of 3-(N-naphthylaminocarbonyl)-2-propenoic acid, HL (Figure 1). These derivatives were characterised by elemental

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analysis, infrared, multinuclear (^{1}H, ^{13}C, ^{119}Sn) NMR and ^{119m}Sn Mössbauer spectroscopy, and mass spectrometry. Biological activities against various fungi and bacteria were tested to establish the significance of the compounds.

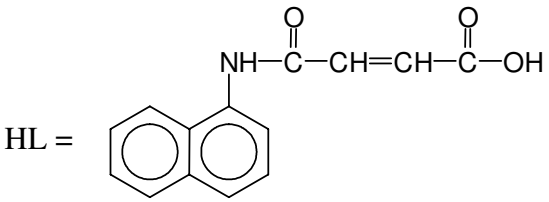


Figure 1. Structure of 3-(N-naphthylaminocarbonyl)-2-propenoic acid, HL.

Results and Discussion

3-(N-naphthylaminocarbonyl)-2-propenoic acid is a fluffy yellow solid with m.p. 147–148 °C. Organotin(IV) derivatives of this acid were prepared by treating the di- and triorganotin(IV) chlorides with the silver salt of the acid in 1:2 and 1:1 molar ratios. The reaction is carried out under an inert atmosphere in anhydrous chloroform. All the synthesised complexes are yellow and are stable in air at room temperature.

All of the investigated compounds were characterised by melting point, elemental analysis and various instrumental techniques. The physical data are reported in Table 1.

Table 1. Physical data of organotin(IV) derivatives of 3-(N-naphthylaminocarbonyl)-2-propenoic acid.

Compound	Formula	Molecular Weight	Yield (%)	Melting Point °C	Elemental Composition		
					% C	**% H**	**% N**
1 n-Bu_2SnL_2	$C_{36}H_{38}O_6N_2Sn$	714	30	120-124	60.50 (61.12)	5.32 (5.20)	3.92 (3.97)
2 Ph_2SnL_2	$C_{40}H_{30}O_6N_2Sn$	754	30	180-182	63.66 (63.95)	3.98 (3.82)	3.71 (3.73)
3 $Benz_2SnL_2$	$C_{42}H_{34}O_6N_2Sn$	782	65	110-112	64.45 (64.52)	4.35 (4.39)	3.58 (3.61)
4 Me_3SnL	$C_{17}H_{19}O_3NSn$	405	70	152-154	50.37 (50.40)	4.69 (4.63)	3.46 (3.52)
5 Bu_3SnL	$C_{26}H_{37}O_3NSn$	531	55	110-111	58.75 (57.00)	6.97 (7.10)	2.63 (2.60)
6 Ph_3SnL	$C_{32}H_{25}O_3NSn$	591	40	207-210	64.97 (65.12)	5.13 (5.23)	2.37 (2.22)
7 $Benz_3SnL$	$C_{35}H_{31}O_3NSn$	633	65	71-72	66.35 (66.19)	4.90 (4.83)	2.21 (2.29)

Infrared spectroscopy

The infrared spectra were recorded in the range 4000-250 cm^{-1} as KBr/CsBr discs and important bands for structural assignments are given in Table 2. In the 3250-2900 cm^{-1} region, the ligand exhibited a medium band typical for -OH stretching vibration. This band was absent from the spectra of the complexes, indicating the coordination of deprotonated carboxylate, -COO^{-} group, to the central tin atom. The ν(Sn-Cl) bands of R_2SnCl_2 and R_3SnCl (350-330 cm^{-1}) were not found in the spectra of the products, indicating complex formation.

Table 2. Characteristic IR absorption bands of organotin(IV) derivatives of 3-(N-naphthyl amino carbonyl)-2-propenoic acid.

Compound	ν(NH-C(O)) cm^{-1}	ν(-COO) cm^{-1}			ν(Sn-C) cm^{-1}	ν(Sn-O) cm^{-1}
		ν_{as}(COO)	ν_s(COO)	$\Delta\nu$		
1	1661	1602	1360	242	553	479
2	1657	1605	1361	244	548	465
3	1653	1612	1358	254	551	475
4	1660	1605	1352	253	560	480
5	1647	1600	1350	250	554	483
6	1658	1604	1352	252	545	453
7	1655	1602	1355	247	540	470
Ligand	1712	1637	1330	307	-	-

The ν(-NH-C(O)-) band, occurring at 1712 cm^{-1} in the spectrum of the ligand, is shifted considerably towards the lower frequencies in the range 1661-1647 cm^{-1}, confirming the one mode of coordination of the ligand with the organotin moiety. The second mode of the coordination of the ligand to organotin moiety is confirmed by the $\Delta\nu$ [ν_{as}(COO) – ν_s(COO)] values, which is very important in determining the mode of the bonding of the carboxylate group, (-COO^-), to the central tin atom[16]. It is generally thought that the difference in $\Delta\nu$ between asymmetric ν_{as}(COO) and symmetric ν_s(COO) absorption frequencies is below 200 cm^{-1} for bidentate carboxylate moiety, but greater than 200 cm^{-1} for monodentate carboxylate moiety. All the values of $\Delta\nu$ of the synthesised compounds are in the range 242–254 cm^{-1} and strongly indicate that the carboxylate group in all compounds adopted a unidentate nature.

^{119m}Sn Mössbauer spectroscopy

The Mössbauer spectra of compounds **4-7** are listed in Table 3. It is clear from the isomer shift (δ) values of the studied compounds that tin is in the +4 oxidation state. The ratio of the quadruple splitting value to the isomer shift value ($\rho = \Delta/\delta$) can be used to distinguish between the different coordination states of the central tin atom[17]. Tin compounds which are 4 coordinated have ρ values less than 1.8, while ρ values larger than 2.1 would indicate compounds with greater than 4 coordination. As can be seen in Table 3, compounds **4-7** have ρ values greater than 2.1, suggesting a coordination number greater than 4. Furthermore, the quadruple splitting values are in the range 3.18–3.55 mms^{-1}, consistent with a *trans*-trigonal bipyramidal geometry with a planar R_3Sn unit, and 2 axial positions are occupied by oxygen atoms of (-NH-C(O)-) and (-COO^-) groups. Further, the quadruple splitting values match closely the values of compounds having a *trans*-trigonal bipyramidal geometry in a polymeric structure[17].

Table 3. ^{119m}Sn Mössbauer parameters of triorganotin(IV) derivatives of 3-(N- naphthylaminocarbonyl)-2-propenoic acid.

Compound	Isomer shift (δ) mms^{-1}	Quadruple splitting (Δ) mms^{-1}	($\rho = \Delta/\delta$) mms^{-1}
4	1.29	3.48	2.70
5	1.41	3.55	2.52
6	1.25	3.18	2.54
7	1.42	3.29	2.31

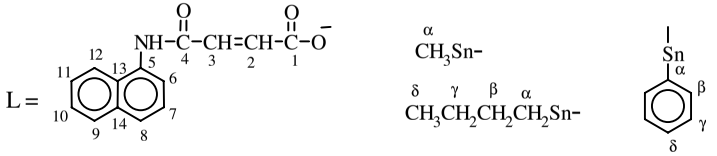
Table 4. ^{1}H NMR data of organotin(IV) derivatives of 3-(N-naphthylamino- carbonyl)-2- propenoic acid [a,b].

^{1}H No.	Compounds							
	Ligand	**1**	**2**	**3**	**4**	**5**	**6**	**7**
2	6.40 d (13.4)	5.93 d (13.2)	6.39 d (13.1)	6.31 d (13.0)	6.34 d (13.1)	6.25 d (13.4)	6.32 d (13.4)	6.27 d (13.4)
3	6.80 d (13.1)	6.28 d (13.2)	6.48 d (13.3)	6.48 d (13.1)	6.37 d (13.4)	6.34 d (13.4)	6.40 d (13.4)	6.46 d (13.4)
6.8,9	7.47-7.56 m	7.06-7.16 m	7.57-7.92 m	6.99-7.10 m	7.44-7.51 m	7.36-7.43 m	7.58-7.72 m	6.99-7.07 m
7	8.08 m	8.07 m	7.57-7.92 m	7.82-7.93 m	8.10-8.21 m	8.11-8.18 m	8.02-8.11 m	7.86 m
10	7.81 m	7.59 m	7.57-7.92 m	7.61 m	7.67 m	7.77 m	7.58 m	7.46 m
11	7.72 m	7.38 m	7.57 m	7.67 m	7.85 m	7.85 m	7.58-7.72 m	7.46-7.51 m
12	7.89 m	7.76 m	7.57 m	7.69 m	7.75 m	7.71 m	7.78 m	7.69 m
NH	8.95 s	8.82 s	8.69 s	8.52 s	8.88 s	9.01 s	8.83 s	8.91 s
CH_2	–	–	–	2.59 s	–	–	–	2.56 s
α	–	1.27 m	7.31-7.51 m	7.20-7.51 m	0.60 s, ^{2}J[56]	1.25 m	7.16-7.44 m	6.71-7.13 m
β	–	1.46 m	–	–	–	1.50 m	–	–
γ	–	1.13 m	–	–	–	1.19 m	–	–
δ	–	0.59 t (7.3)	–	–	–	0.77 t (7.2)	–	–

[a] Chemical shift (δ) in ppm, $^{3}J(^{1}H-^{1}H)$ and $^{2}J[^{119/117}Sn-^{1}H]$ in Hz.

[b] Multiplicity is given as s = singlet, d = doublet, t = triplet, m = multiplet

[c] For number and α, β and γ see structures below.



Multinuclear NMR spectroscopy

^{1}H NMR data for compounds **1-7** are given in Table 4. The expected resonances are assigned based on their multiplicity and intensity pattern. For the trimethyltin derivative (**4**), the $^2J(^{119}\text{Sn},^1\text{H})$ value is 56 Hz, which falls in range of the tetrahedral environment around the tin atom[7]. In the case of butyl and phenyl derivatives, the $^nJ(^{119}\text{Sn},^1\text{H})$ couplings are not visible due to a complex multiple pattern. Table 5 presents the ^{13}C NMR and ^{119}Sn NMR data for the synthesised organotin compounds. The magnitudes for $^nJ(^{119}\text{Sn},^{13}\text{C})$ couplings are also observed for compounds **4** and **5**. The magnitudes of $^1J(^{119}\text{Sn},^{13}\text{C})$ couplings suggest the typical tetrahedral geometry around tin in solution[7]. As far as the geometry of the diorganotin dicarboxylates (**1-3**) in non-coordinated solvents is concerned, it is not defined with certainty due to the fluxional behaviour of the carboxylate oxygens in their coordination with the tin atom. However, earlier reports suggest geometry between penta- and hexa-coordination[7]. ^{119}Sn NMR is also a powerful technique for the determination of the coordination number of tin[18]. Generally triorganotin carboxylates, $R_3SnOCOR^1$, are known to adopt a variety of motifs in the solid state[3,6]. However, in solution, such structures appear as 4-coordinate, the additional coordination from the carbonyl oxygen to tin being lost[7]. In similar fashion, the δ^{119}Sn chemical shifts of compounds **4-7** are comparable with earlier reports describing tetrahedral geometry[7,8].

Table 5. ^{13}C and ^{119}SnNMR data of organotin(IV) derivatives of 3-(N-naphthyl- aminocarbonyl)-2-propenoic acid[a,b].

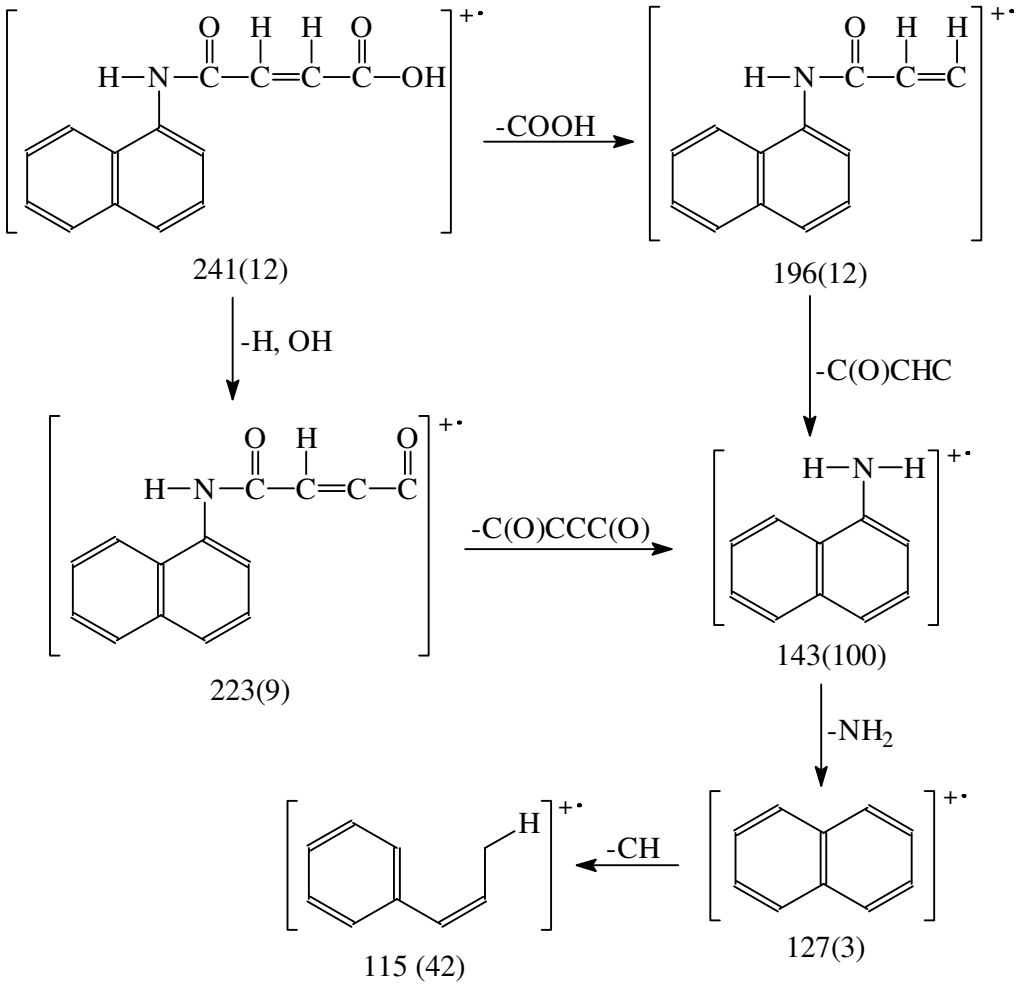
^{13}C No.	Compounds							
	Ligand	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	165.3	162.6	162.8	167.5	163.0	163.0	162.7	162.8
2	126.5	125.5	126.2	126.7	125.7	125.7	125.8	125.0
3	127.0	125.8	126.5	127.2	125.8	125.8	125.9	125.5
4	175.3	171.3	171.7	175.1	171.2	171.3	171.4	171.6
5	136.9	138.0	138.5	136.5	138.2	138.3	137.9	137.9
6	120.9	119.8	121.1	121.6	120.3	119.8	119.9	120.1
7	128.8	129.0	128.5	129.7	128.6	128.4	128.2	128.6
8	122.6	121.2	121.3	122.9	121.9	121.8	121.7	121.6
9	130.1	132.7	130.6	130.8	133.0	133.2	130.6	130.1
10	128.5	128.7	128.6	128.9	128.4	128.3	128.5	128.1
11	128.1	128.2	127.4	128.2	127.2	127.0	127.3	127.2
12	125.4	125.3	125.4	125.6	125.4	125.2	125.3	124.8
13	127.5	126.7	127.9	127.9	125.9	126.0	125.7	126.1
14	131.9	135.0	134.0	134.3	134.1	134.1	134.1	134.2
CH_2				30.0				24.5
α	–	18.5	136.7	124.1	-1.8, ^{1}J[405, 387]	17.1, ^{1}J[347, 337]	138.8	124.3
β	–	27.4	136.3	128.0	–	27.7	136.7	128.9
γ	–	27.1	128.0	127.6	–	27.0, ^{3}J[66]	129.1	127.6
δ	–	13.6	130.0	125.0	–	13.6, ^{4}J[34]	130.0	125.8
δ^{119} Sn	-	-	-	-	149.5	135.4	-87.9	6.7

[a] Chemical shift (δ) in ppm, $^1J[^{119/117}\text{Sn}-^{13}\text{C}]$ in Hz

[b] For numbering see footnotes of Table 4

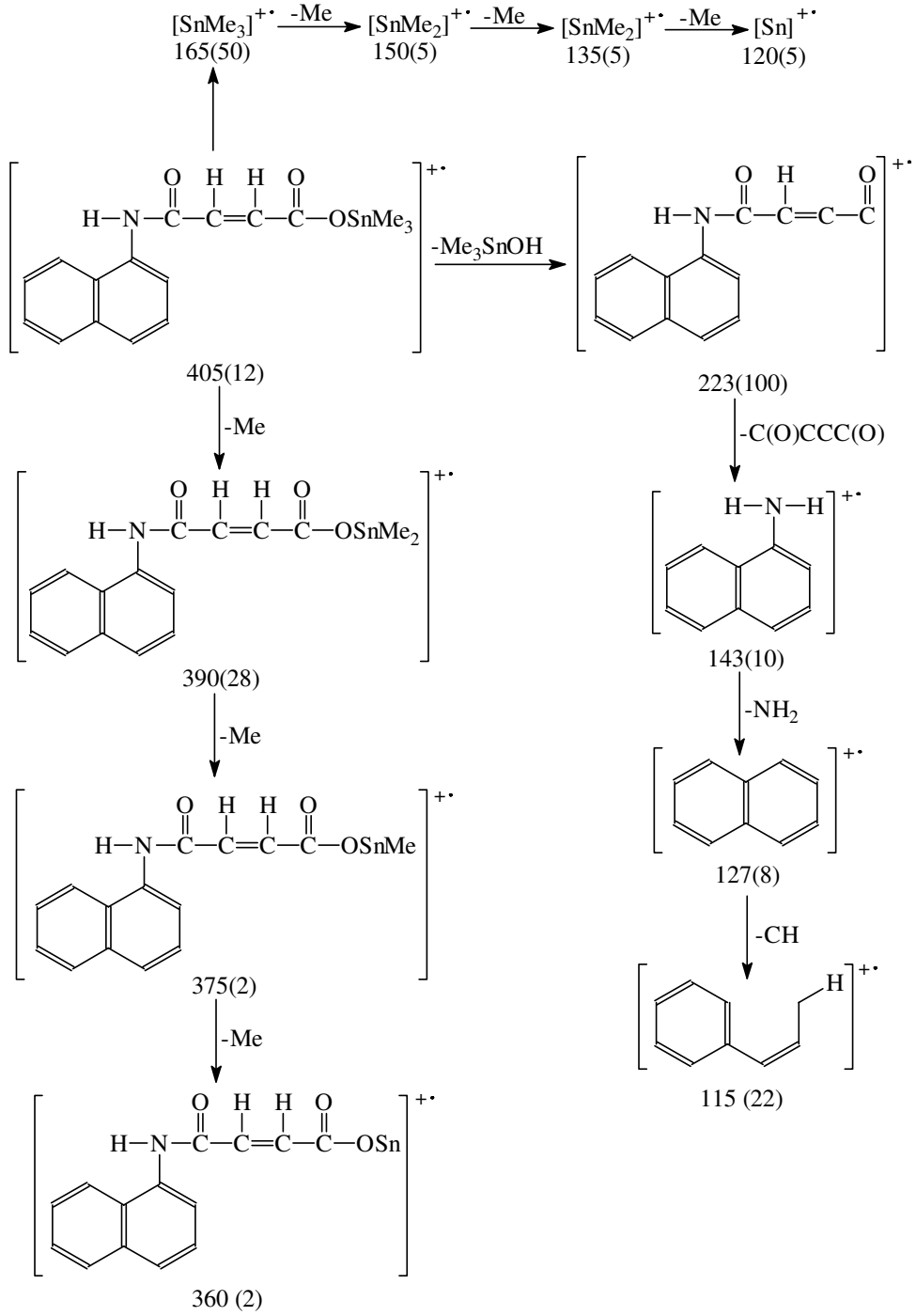
Mass spectrometry

The conventional EI mass spectral data for ligand acid and its organotin derivatives were recorded and different fragmentation patterns for the ligand and one of its representatives were proposed and are listed in the Schemes 1 and 2 along with m/z and % intensity. The molecular ion peak M^+ for ligand acid appeared at m/z 241 with 12% intensity. Loss of different groups like H, OH, COOH, C(O)CCC(O) and C(O)CHC results in different peaks of respective m/z, which on further loss gave a peak at m/z 143 (100) as the base peak for the positive ion $C_{10}H_7NH_2$. The further elimination of NH_2 and CH group produced the fragment ion $[C_6H_4CHCHCH]^+$ at m/z 115 (42). The successive loss of the CH group resulted in $[C_6H_5]^+$, $[C_6H_4]^+$and $[C_6H_3]^+$ fragment ions at m/z 77, 76 and 75, respectively. In mass spectral data of tri- and diorganotin(IV) derivatives, each fragment ion occurs in a group of peaks as a result of tin isotopes. For simplicity the mass spectral fragmentation data reported here are related to the principal isotope ^{120}Sn [19]. The molecular ion peaks $[M]^+$were observed in compounds **4** and **5** only. Scheme 2 presents the fragmentation pattern of compound **4**. Three primary fragmentation patterns are proposed, based on observed m/z in the mass spectrum of compound **4**. Elimination of the Me_3SnOH group gave $[C_{10}H_7NHC(O)CHCCO]^+$ at m/z 223



Scheme 1

as a base peak, which further eliminates groups as mentioned in Scheme 1. The second pathway after primary elimination of the $[C_{10}H_7NHC(O)CHCCO]^+$ group results in the formation of $[Sn]^+$. The third pathway involves the successive elimination of the $[CH_3]^+$ group from the molecular ion $[M]^+$ peak. The different fragmentation patterns proposed for the representative compound fully support the fragmentation for the reported organotin compounds and related compounds reported in the literature[20].



Scheme 2

Biological activity

Table 6 presents the antifungal activity of 3-(N-naphthylaminocarbonyl)-2-propenoic acid and its di- and triorganotin(IV) derivatives. The evaluation of the data reveals that generally the fungal growth inhibition activity of the triorganotin compounds is higher than that of the diorganotin compounds and ligand acid. Compounds **4** and **5** show the highest antifungal activity, while the antifungal activity of compound **3** is the lowest.

Table 6. Antifungal data of organotin(IV) derivatives of 3-(N-naphthylaminocarbonyl)-2-propenoic acid.

Fungus	Percent Inhibition								Standard Inhibition	Percent
	Ligand	**1**	**2**	**3**	**4**	**5**	**6**	**7**		
Trichophyton longiformis	45.3	60	89	50	100	100	89	75	Miconazole Ketoconazole	100
Candida albicans	50.5	80	100	55.5	100	100	100	95	Miconazole Ketoconazole	100
Aspergillus flavis	40.0	65	100	40.3	100	100	100	85	Amphotericin. B Flucytosine	100
Microsporum canis	50.8	80	88.2	60.0	100	100	88.2	85	Miconazole Ketoconazole	100
Fusarium solani	60.2	30	94.1	35.0	100	100	94.2	83	Benlate Naban	100
Fusarium moniliformis	20.0	25	71.4	10.0	100	100	85.7	54	Benlate Naban	100

Antibacterial activity tests of the ligand and its derivatives were carried out against a set of bacteria by the agar well diffusion method[21]. The results are shown in Table 7. It is clear from the tabulated values that the antibacterial activity of the ligand is markedly low as compared to the standard reference drug. Generally, the antibacterial activity of the synthesised compounds is higher than that of the ligand and lower than that of the reference drug. Compounds **2**, **5** and **6** inhibit the growth of *Corynebacterium diphtheriae*, *Bacillus subtilis, Streptococcus pyogenes* and *Staphylococcus aureus* excellently.

Table 7. Antibacterial data of organotin(IV) derivatives of 3-(N-naphthylamino- carbonyl)-2-propenoic acid.

Bacterium	Zone of Inhibition (mm)								Reference Drug*
	Ligand	**1**	**2**	**3**	**4**	**5**	**6**	**7**	
Corynebacterium diphtheriae	16	27	28	18	22	27	29	22	34
Bacillus subtilis	17	26	28	19	20	28	28	23	30
Streptococcus pyogenes	15	24	26	18	21	28	27	23	30
Staphylococcus aureus	14	26	26	18	20	24	29	24	30
Pseudomonas aeruginosa	18	19	19	16	20	18	17	20	26
Salmonella typhi	16	19	19	20	19	19	19	16	38

*Tetracycline

Cytotoxic data of the ligand acid and its derivatives were collected by the brine-shrimp lethality bioassay method[22] and the results are given in Table 8. The evaluation of the data reveals that LD_{50} values of compound **2** are comparable with that of the ligand acid. Compounds **6** and **7** are more toxic, while compounds **1** and **5** are less toxic than the parent ligand acid. Compounds **3** and **4** are the least toxic among all the studied di- and triorganotin(IV) derivatives of 3-(N-naphthylaminocarbonyl)-2-propenoic acid.

Table 8. Cytotoxicity data[a] of organotin(IV) derivatives of 3-(N-naphthylamino- carbonyl)-2-propenoic acid.

Compound	Upper Toxic Conc.	Toxicity LD_{50} (μg/mL)	Lower Toxic Conc.
Ligand	-	0.19	-
1	2.79	1.53	0.61
2	0.19	0.16	0.13
3	12.98	7.77	4.70
4	16.93	10.42	6.31
5	1.20	0.60	0.46
6	0.08	0.06	0.05
7	0.12	0.10	0.07

[a] Against Brine-shrimp (in vitro)

Experimental

Materials and instrumentation

All the chemicals were of analytical grade and were used without further purification. Tri and diorganotin chlorides were procured from Aldrich and Fluka except for di- and tribenzyltin chlorides, which were prepared by the reported method[23]. Organic solvents were dried prior to use according to the standard procedure[24].

Melting points were determined in a capillary tube using electrothermal melting point apparatus model MPD Mitamura Riken Kogyo (Japan) and are uncorrected. The elemental analyses were performed on an Organic Elemental Analyser model EA 1110, CE Instrument, Italy. Infrared spectra within the range 4000-400 cm^{-1}were recorded in KBr / CsI pellets on a Perkin Elmer FT-IR Spectrometer model Spectrum 1000. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 250 Spectrometer (Germany), using $CDCl_3$ as an internal reference [$\delta^{1}H(CDCl_3 = 7.24 : \delta^{13}C(CDCl_3) = 77.0]$. ^{119}Sn NMR spectra were obtained with Me_4Sn as external reference [Ξ (Sn) = 37.290665]. ^{119m}Sn Mössbauer spectra were obtained with a constant acceleration microprocessor controlled spectrometer (Cryoscopic Ltd., Oxford, UK); a barium stannate source was used at room temperature, and samples were packed in Perspex discs and cooled to 77 K. Isomer shift data are relative to SnO_2. Mass spectral data were measured on a MAT 8500 Finnigan 70 eV mass spectrometer (Germany). The m/z values were evaluated assuming that H =1, C = 12, N = 14 and Sn = 120.

Synthesis of ligand

3-(N-naphthylaminocarbonyl)-2-propenoic acid was prepared by adding 0.06 mol (8.59 g) of 1−naphthylamine to a solution containing an equal molar quantity (0.06 mol, 5.88 g) of maleic anhydride in 100 mL of glacial acetic acid. The reaction mixture was stirred and a yellow solid began to appear. After 1 h, precipitation

was completed by dilution with 1 L of water. A yellow product was filtered, washed with water, dried and recrystallised from ethanol and water.

Synthesis of organotin compounds

The silver salt of 3−(N−naphthylaminocarbonyl)−2−propenoic acid was prepared by dissolving 0.02 mol (4.82 g) of acid in 200 mL of ethanol and adding it to a solution containing an equal molar quantity (0.02 mol, 1.68 g) of sodium bicarbonate. A solution of silver nitrate (0.02 mol, 3.397 g) was added dropwise to the above mixture. The resulting precipitates were filtered, washed with water and dried over anhydrous calcium chloride in the dark.

Then 0.01 mol (3.478 g) of the silver salt of the acid was suspended in 100 mL of dry chloroform in a 250 mL 2-neck round bottom flask equipped with a magnetic stirring bar and a condenser. Diorganotin(IV) dichlorides (0.005 mol)/triorganotin(IV) chlorides (0.01 mol) were slowly added with constant stirring to the above salt mixture. The reaction mixture was refluxed for 8 h in an inert atmosphere. The silver chloride formed during the reaction was removed by filtration and the solvent was evaporated under reduced pressure to obtain compounds of type **a** and type **b**. These compounds were recrystallised from a chloroform/n–hexane (1:1) mixture.

Type "a" R_2SnL_2

a	(**1**)	(**2**)	(**3**)
R	n–C_4H_9	C_6H_5	$C_6H_5CH_2$

Type "b" R_3SnL

b	(**4**)	(**5**)	(**6**)	(**7**)
R	CH_3	n–C_4H_9	C_6H_5	$C_6H_5CH_2$

Antifungal activity

The synthesised compounds were also tested for antifungal activity against 6 different human, animal and plant pathogens, namely *Trichophyton longiformis, Candida albicans, Aspergillus flavis, Microsporum canis, Fusarium solani,* and *Fusarium moniliformis* using a tube diffusion test[21]. The Micoanazole, Ketocanozole, Amphotericine B and Flucytosine (75 μg/L) and Benlate and Nabam (50 μg/mL) were used as standard drugs. Stock solutions of pure compounds (12 μg/mL) were prepared in sterile DMSO. Sabouraud dextrose agar was prepared by mixing Sabouraud (32.5 g), glucose agar (4%) and agar-agar (4 g) in 500 mL of distilled water, followed by steamed dissolution, and 4 mL media was dispensed into screw-capped tubes and autoclaved at 121 °C for 15 min. Test compound (66.6 μL) was added from the stock solution to non-solidified Sabouraud agar media (50 °C). The tubes were allowed to solidify at room temperature and inoculated with 4 mm diameter of inocula derived from a 7-day-old respective fungal culture. For non-mycelial growth, an agar surface streak was employed. The tubes were incubated at 27-29 °C for 7-10 days and the growth in the compound-containing media was determined by measuring the linear growth (mm) and growth inhibition with reference to the respective control. The results of antifungal activity are shown in Table 6.

Antibacterial activity

The synthesised compounds were screened for antibacterial activity against *Corynebacterium diptheriae, Bacillus subtilis, Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeroginosa* and *Salmonella typhi* bacterial strains using agar well diffusion[21]. Tetracycline was used as the standard drug. The wells were dug in the media with the help of a sterile metallic borer with centres at least 24 mm apart. Bacterial inocula 2-8 h old containing approximately 10^4-10^6 colony forming units (CFU)/mL were spread on the surface of nutrient agar with the help of a sterile cotton swab. The recommended concentration of the test sample (1 mg/mL in DMSO) was introduced into respective wells. The other wells were supplemented with DMSO and reference antibacterial drugs, serving as negative and positive controls, respectively. The plates were incubated immediately at 37 °C for 20 h. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was calculated with reference to the positive controls. The results of antibacterial activity are collected in Table 7. LD_{50} values were also determined by a brine-shrimp method[22] and are reported in Table 7.

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