Enzyme Inhibition Effect and Solid-State Spectral Analysis of Biologically Active Diorganotin(IV) Esters of N-Maleoy-L-Alanine

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ABSTRACT

Enzyme inhibiting activity of five diorganotin(IV) esters of N-maleoyl-L-alanine has been reported for the first time. These compounds have also been tested *in vitro* for insecticidal and anti-tumour activity and significant results were exhibited during these screenings. The synthesized complexes were characterized by means of different solid-state spectroscopic techniques including FT IR, ^{119m}Sn Mössbauer, multinuclear NMR (^{1}H, ^{13}C, ^{119}Sn) and thermogravimetric analysis (TGA). The spectroscopic data proved the hyper-coordination of Sn(IV) atom and an octahedral geometry has been assigned to all the complexes in the solid-state. Elemental analysis data supports the spectroscopic evidence. Energy of activation, molecularity and order of reaction of the title complexes have also been determined, based on the data obtained from TG measurements.

INTRODUCTION

Organotin(IV) compounds are the subject of interest for the last few decades because of their wide range of daily life practical applications and pharmaceutical potential /1-4/. There are environmental concerns over tributyltin(IV) compounds beyond concentration of a few ppm; but diorganotin(IV) compounds are the main focus of interest as future's anti-tumour drugs /5-7/. Many research groups are actively working on the pharmaceutical prospects of organotin(IV) esters of carboxylic acids of biological significance /8-14/. Recently, we have reported some pharmacologically important organotin(IV) complexes of N-maloeoyl protected amino acids /15-21/. We were of the view that it would be interesting to synthesize organotin(IV) derivatives of N-maleoyl-L-alanine (enzyme inhibitor) /22/; study the solid-state structural properties and enzyme inhibition; moreover, we also carried out the *in vitro* insecticidal and anti-tumour activity for the appraisal of their bioavailability.

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EXPERIMENTAL

Materials

Maleic anhydride, L-alanine, triethylamine, dimethyltin(IV) dichloride, diethyltin(IV) dichloride, dipropyltin(IV) dichloride and dibutyltin(IV) dichloride of analytical purity were procured from Aldrich and used as such, while dibenzyltin(IV) dichloride was prepared as reported /23/. Solvents used during this work were dried using standard procedures /24/.

Instrumentation

Elemental analyses (C, H, N) were performed on a Yanaco high-speed CHN analyzer; antipyrene was used as a reference, while tin content was estimated according to reported procedures /25/. Uncorrected melting point was taken on a Reichert Thermovar of F. G. Bode Co., Austria.

The FT-IR spectra of the pure solid samples were recorded on Bruker FT-IR spectrophotometer TENSOR27 (ZnSe), using OPUS software covering 5000-400 cmP^{-1P}.

For Mössbauer measurements, the solid samples were maintained in liquid nitrogen at temperature 77.3 K, V.G. Micromass 7070 F Cryolid liquid nitrogen cryostat. The multichannel calibration was performed with an enriched iron foil using ^{57}Co-Pd source, while the zero point of the Doppler velocity scale was determined through the absorption spectra of $CaSnO_3$ (^{119}Sn = 0.5 mg cm^{-2}). The resulting 5 x 105 –count spectra were refined to obtain the isomeric shift, IS (mm s^{-1}), the nuclear quadrupole splitting QS, ρ (mm s^{-1}) and the width at half-height of the resonant peaks, Γ (mm s^{1}).

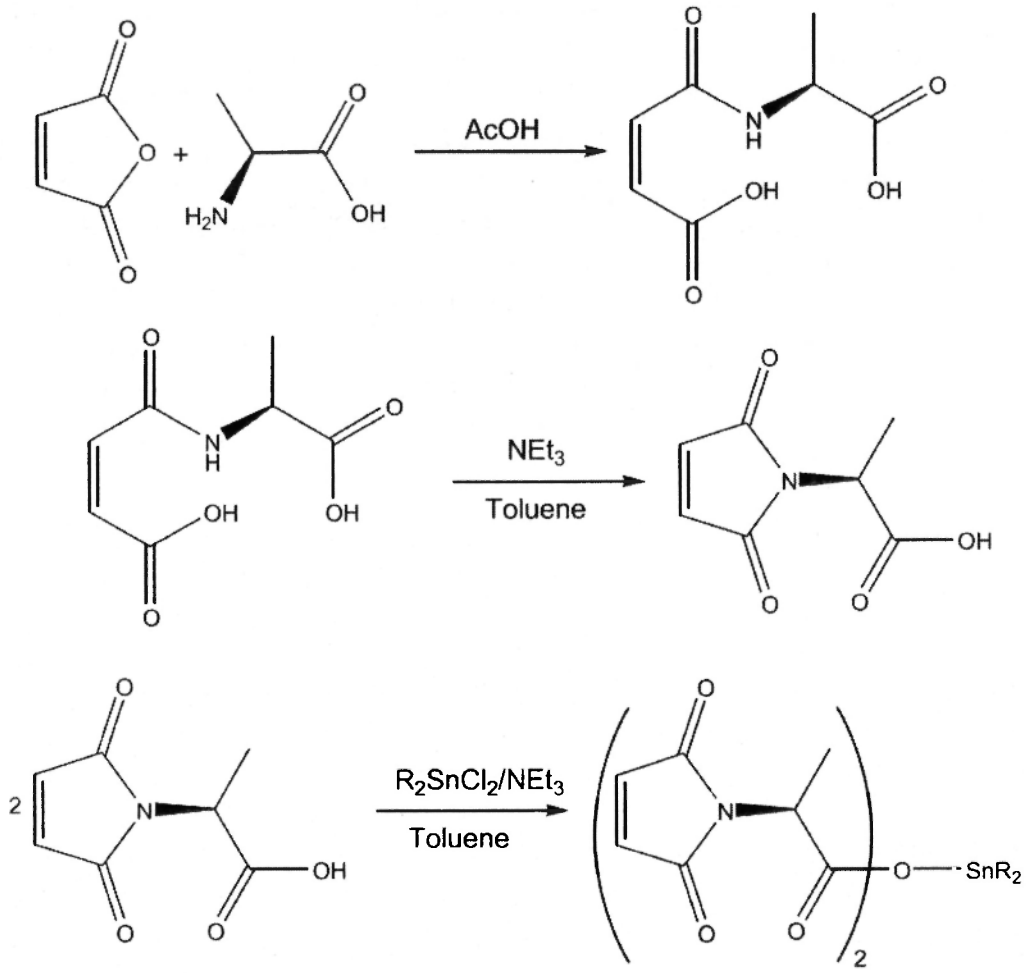
The solid-state ^{1}H NMR spectra were run on a Bruker DMX-300 NMR spectrometer (B_o = 7 T), equipped with a Bruker CPMAS probe with Larmor frequency of 300 MHz. 10 mg of the complexes was packed in 7 mm MAS rotor with spherical spacer and spun at 4.5 KHz. The solid-state ^{13}C and ^{119}Sn NMR spectra of the studied complexes were obtained at 50.32 MHz and 74.63 MHz respectively on a Bruker DSX 200 spectrometer equipped with a double-bearing CP/MAS probe at room temperature. The complexes were packed in standard 4 mm or 7 mm ZrO_2 rotor. The ^{13}C and ^{119}Sn Hartmann-Hahn cross-polarization match was set with adamantane and tetracyclohexyltin respectively, using a ^{1}H 90° pulse of 4 μs. Contact time was set to 1-2 ms. Recycle delay was 10 second. In the case of ^{119}Sn CP/MAS NMR experiments, at least two spinning frequencies (4.5-9 KHz) were used to identify the isotropic chemical shift. The number of scans varied between 200 and 22000 to achieve acceptable signal-to-noise ratios. The ^{13}C and ^{119}Sn chemical shifts were calibrated indirectly by external glycine (carbonyl signal δ = 176.03 ppm) and tetracyclohexyltin (δ = −97.35 ppm) respectively.

Thermoanalytical measurements were carried out using a Perkin Elmer Thermogravimetric/Differential Thermal Analyzer (YRIS Diamond TG-DTA High Temp. Vacu.) consuming variable heating rates between 5 °C/min and 10 °C/min in inert atmosphere.

METHODS

Synthesis of 2-maleimidopropanoic acid

Maleic anhydride (10 g, 101.9782 mM) was dissolved in acetic acid (150 ml) and a cold solution of L-alanine (9.0852 g, 101.9782 mM) in acetic acid (150 ml) was added. This mixture was stirred at room temperature for 3 hours resulting in a white precipitate. This precipitate was washed thrice with cold water and recrystallized from water to get maleamic acid of analytical purity. Maleamic acid (5 g, 26.7165 mM) was suspended in dry toluene (350 ml) and triethylamine (7.4806 ml, 53.4330 mM) was added to this suspension and refluxed with vigorous stirring for 1.5 hours, with concomitant removal of water using a Dean-Stark separator. The solvent was removed on a rotary evaporator (Büchi) leaving a hygroscopic solid; HCl was added up to pH 2, the product extracted with ethyl acetate and dried over anhydrous $MgSOB_{4B}$. The ethyl acetate fraction was vacuum dried; the solid mass left was recrystallized from hexane and a general chemical reaction scheme is given as Scheme I.



Scheme I

Synthesis of organotin(IV) complexes of N-maleoyl-L-alanine

A solution of triethylammonium salt of N-maleoyl-L-alanine (0.5 g, 2.9563 mM) in dry toluene (75 ml) was prepared and appropriate amount of diorganotin(IV) dichloride (2.9563 mM) was added. This mixture was heated to reflux for 3 hours, which resulted in turbidity due to the formation of triethylammoniumhydrochloride; this was filtered off and the filtrate was evaporated on a rotary evaporator. The solid mass was dissolved in a mixture of C_6H_6 and CH_2Cl_2 (1:1) and finally the complex was recrystallized from CH_2Cl_2 (Scheme I).

In vitro anti-tumour activity on human tumourai cell lines

The *in vitro* inhibition concentrations (IC_{50} ng/ml) are presented in Table 3. The complexes **1-5** and the ligand **6** were screened *in vitro* against seven human cancer cell lines i.e. H157 and H1299 lung cancers, SK-CO-1 and SW403 colon cancers and HT1376 bladder cancer. Reference drugs used were adriamycin, mitomycin C and vinblastine, using Hoechst 33258 fluorescence protocol that measures the metabolic activity of living cells. In brief, the cells were seeded onto 96-well microtiter plates at a concentration of $5 \times 10^4 - 10^5$ cells ml^{-1}, volume 200 µl/well. Medium used was RPMI-1640 buffered with 2.2 g/l $NaHCO_3$, supplemented with 10% heat-inactivated foetal bovine serum (HIFBS); pH 7.4. The plates were incubated at 36.5 °C in a humidified CO_2 (10%) incubator for 24 h. Fresh medium was added to remove old medium. 10 µl containing different concentrations of test compounds were added, whereas +ve and –ve controls had standard drugs and no drug respectively. Then, these plates were incubated for the next 48-72 h at 36.5 °C in a humidified CO_2 (10%) incubator. After incubation, the medium was removed from the wells, plates were kept at -80 °C for 1-2 hr. and thawed at 50 °C for 15 minutes. To each well, 100 µl distilled water was added and the plates were incubated at room temperature. 0.1 ml of TNE containing 20 µl/ml Hoechst 3325 dye was added and mixed well on a plate shaker. The plates were incubated in dark for 1.5 hr. at room temperature. The fluorescence was measured at 350/460 nm in a fluorescent plate and ID_{50} values for compounds **1-6** possessing cytotoxic activity were calculated.

In vitro insecticidal activity

Contact toxicity insecticidal assay was used to assess the direct insecticidal action of **1-6** against *Pectinophora gosspiella* (Saunders), *Gelechiidae* (serious pest of cotton) and *Noctuidae* (common crop pest). Concentrations of 250, 200, 100 and 50 µg of each compound were dissolved in chloroform and coated on the inner surface of 20 ml glass vials. Every vial was hand-rotated until the solution was distributed on the vial inner wall and floor and till evaporation of the sample. Then each vial was placed in a fume hood for 15 minutes to ensure complete removal of chloroform. Five insects were carefully placed in each vial with sufficient food. The survival of the insects was assessed for 24-48 hours. Positive control consisted of test insects in vials treated only with the carrier solvent (survival averaged > 97%); negative control consisted of commercially available insecticide Mortein. Every compound was run in triplicate. Percent survival rate was

calculated as: Survival rate (%) = No. of insects survived / Total no. of insects x 100

Urease assay and inhibition

Reaction mixtures comprising 25 µl of enzyme (Jack bean and Bacillus pasteurii Urease) solution were incubated for 30 minutes with µl test compound (**1-6**) at 30 °C for 15 minutes in 96-well plates and then 55 µl of buffers containing 100 mM urea were incubated for 15 min. At the end final urease activity was determined by measuring ammonia production using the indophenol method as described by Weathburn []. Briefly, 45 µl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µl of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using microplate reader (Molecular Device, USA). All the reactions were performed in triplicate in a final volume 200 µl. The results were processed by using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_43H_2O$, ImM EDTA and 0.01 M LiCl). Percentage inhibitions were calculated from the formula $100 - [(OD_{testwell} / OD_{control}) \times 100]$. Thiourea was used as the standard inhibitor of urease.

Spectral and analytical data

Abbreviations: Yield: %; Mp: melting point; Elemental analysis: %; ν: IR vibration in cm^{-1}; QS: quadropole splitting, IS: isomeric shift, Γ_1 and Γ_2: line-widths (all in mm s^{-1}), ρ: QS/IS; δ: Chemical shift in parts per million (ppm) downfield from the standard; *J*, coupling constant in hertz; multiplicities, s: singlet, d: doublet, t: triplet, q: quartet; m: multiplet; Ea: Energy of activation; Ro: Reaction order;

Me_2SnR_2(**1**)

Mp 158 °C; Yield: 81 %; Anal. calcd. for $C_{16}H_{18}N_2O_8Sn$: C: 39.62, H: 3.74, N: 5.78, Sn: 24.47; Found: C: 39.45, H: 3.56, N: 5.63, Sn: 24.28; $\nu(COO)_{asym}$: 1625, $\nu(COO)_{sym}$: 1440, Δν: 185, $\nu(Sn—C)_{asym}$: 513, $\nu(Sn—C)_{sym}$: 503, ν(Sn—O): 466; QS: 3.42, IS: 1.21, Γ_1: 0.99, Γ_1: 0.97, ρ(QS/IS). 2.82; ^{1}H δ: 2H, 4.58q; 3H, 1.66d; 5H, 7.11s; 6H, 0.13s (^2J: 103); ^{13}C δ: C1, 179.98; C2, 55.04; C3, 18.33, C4, 171.12; C5, 1318.65; C6, 0.14 (^1J: 942).

Et_2SnR_2(**2**)

Mp 74 °C; Yield: 77 %; Anal. calcd. for $C_{18}H_{22}N_2O_8Sn$: C: 42.14, H: 4.32, N: 5.46, Sn: 23.14; Found: C: 41.97, H: 4.21, N: 5.31, Sn: 23.01; $\nu(COO)_{asym}$: 1643, $\nu(COO)_{sym}$: 1480, Δν: 163, $\nu(Sn—C)_{asym}$: 510, $\nu(Sn—C)_{sym}$: 502, ν(Sn—O): 442; QS: 3.55, IS: 1.07, Γ_1: 0.98, Γ_1: 0.89, ρ(QS/IS): 3.31; ^{1}H δ: 2H, 4.74q; 3H, 1.77d; 5H, 7.32s; 6H, 0.88q (^2J: 99); 7H, 0.72t; ^{13}C δ: C1, 170.25; C2, 53.58; C3, 17.14; C4, 174.61; C5, 139.05; C6, 11.08 (^1J: 925); C7, 6.51 (^3J: 128).

Pr_2SnR_2(**3**)

Mp 113 °C; Yield: 89 %; Anal. calcd. for $C_{20}H_{26}N_2O_8Sn$: C: 44.39, H: 4.84, N: 5.18, Sn: 21.94; Found: C: 44.23, H: 4.79, N: 5.03, Sn: 21.72; $\nu(COO)_{asym}$: 1580, $\nu(COO)_{sym}$: 1431, Δν: 149, $\nu(Sn—C)_{asym}$: 509, $\nu(Sn—C)_{sym}$: 500, ν(Sn—O): 452; QS: 3.14, IS: 1.19, Γ_1: 0.91, Γ_1: 0.96, ρ(QS/IS): 2.64; ^{1}H δ: 2H, 4.69q; 3H, 1.54d; 5H, 7.20s; 6H, 0.99t (^2J: 102); 7H, 1.68m; 8H, 1.15t; ^{13}C δ: C1, 170.21; C2, 53.22; C3, 18.09;

C4, 171.04; C5, 136.96; C6, 29.42 (^1J: 932); C7, 21.33 (^2J: 124); C8, 23.74 (^3J: 201).

Bu_2SnR_2(**4**)

Mp > 350 °C; Yield: 83 %; Anal. calcd. for $C_{22}H_{30}N_2O_8Sn$: C: 46.42, H: 5.31, N: 4.92, Sn: 20.86; Found: C: 46.25, H: 5.26, N: 4.84, Sn: 20.61; $\nu(COO)_{asym}$: 1605, $\nu(COO)_{sym}$: 1445, $\Delta\nu$: 160, $\nu(Sn—C)_{asym}$: 535, $\nu(Sn—C)_{sym}$: 509, $\nu(Sn—O)$: 497; QS: 3.69, IS: 1.17, Γ_1: 0.92, Γ_1: 0.87, ρ(QS/IS): 3.15; ^{1}H δ: 2H, 4.63q; 3H, 1.62d; 5H, 7.25s; 6H, 1.02t (^2J: 100); 7H, 1.54m; 8H, 1.21m; 9H, 0.81t; ^{13}C δ: C1, 172.40; C2, 54.13; C3, 19.14; C4, 172.66; C5, 138.38; C6, 28.31 (^1J: 920); C7, 25.07 (^2J: 124); C8, 23.14 (^3J: 201); C9, 13.87 (^4J: 187).

Bz_2SnR_2(**5**)

Mp 93 °C; Yield: 88 %; Anal. calcd. for $C_{28}H_{26}N_2O_8Sn$: C: 52.78, H: 4.11, N: 4.40, Sn: 18.63; Found: C: 52.67, H: 4.03, N: 4.26, Sn: 18.39; $\nu(COO)_{asym}$: 1591, $\nu(COO)_{sym}$: 1433, $\Delta\nu$: 158, $\nu(Sn—C)_{asym}$: 550, $\nu(Sn—C)_{sym}$: 508, $\nu(Sn—O)$: 481; QS: 3.63, IS: 1.33, Γ_1: 0.95, Γ_1: 0.90, ρ(QS/IS): 2.72; ^{1}H NMR δ: 2H, 4.63q; 3H, 1.74d; 5H, 7.25s; 6H, 2.88s (^2J: 101); 8H, 7.14m; 9H, 7.45m; 10H, 7.22m; ^{13}C δ: C1, 173.28; C2, 53.45; C3, 16.14; C4, 173.49; C5, 139.22; C6, 20.17 (^1J: 916); C7, 142.0 (^2J: 174); C8, 129.3 (^3J: 223); C9, 133.1 (^4J: 231); C10, 128.2.

RESULTS AND DISCUSSION

The complexes and the ligand were synthesized by a general procedure depicted in Scheme I. Analytical data for the complexes showed metal/ligand 1:2 stoichiometry. All the compounds were quite stable with good yield (77-88%) and are soluble in most organic solvents. Elemental analysis data found were in good agreement with those of calculated ones. The physical and analytical data for the investigated compounds are reported in Table 1.

Spectroscopic results

IR spectra were recorded in the spectral range of 4000-400 cm^{-1}.The important frequencies are ν_{asym}(COO), ν_{sym}(COO), ν_{asym}(Sn-C), ν_{sym}(Sn-C) and ν_{asym}(Sn-O). The formation of complexes between R_2Sn(IV) moieties and N-maleoy-L-alanine was confirmed by absence of a broad band of ν(OH) of COOH group in the spectral range of 3000-2600 cm^{-1} /26/. The band in the range of 1700-1710 cm^{-1} for imide ν(N-C=O) remained unchanged, which ruled out the interaction of Sn with imide CO /7-9/. The difference between ν_{asym}(COO) and ν_{sym}(COO) is important in forecasting the denticity of the ligand; in **1-5**, the difference ($\Delta\nu$) was less than 200 cm^{-1}, which indicated the bidentate nature of N-maleoy-L-alanine (Fig. 1) /27-29/. Moreover, bands of medium intensity in the spectral range of 570-545 cm^{-1} and 490-430 cm^{-1} confirmed the presence of Sn-C {ν_{asym}(Sn-C), ν_{sym}(Sn-C)} and Sn-O bonds respectively.

^{119m}Sn Mössbauer spectroscopy provides useful information on the geometry around tin atom in the solid-state /30-32/. In particular quadropole splitting (QS) values often allow the discrimination of tetra- and hyper-coordination of Sn(IV) centre, each of these being identified by characteristic value range (tetrahedral: 20.1-

2.5 mms^{-1}, trigonalbipyramidal: 3.0-4.0 mms^{-1}, cis-octahedral: 1.7-2.2 mms^{-1}, trans-octahedral: 3.5-4.5 3.0-4.0 mms^{-1}) [31-33]. For diorganotin(IV) dicarboxylates, the ρ values (QS/IS) plays an important role in the prediction of the geometry around the tin atom; literature revealed that if the ρ value is greater than 2.1, the dirganotin(IV) dicarboxylates possesses a trans-octahedral geometry around the tin atom; hence ρ values strongly suggested a trans-octahedral geometry for **1-5** (Fig. 1) /34-36/.

Table 1

Thermal decomposition data and kinetic parameters of **1-5**.

Compound	Ts (°C)	Weight loss % Calcd.	Weight loss % Obs.	Error	n	Ea I (kcal/mol)	Ea I (kcal/mol)
	230	23.56	23.12	0.44	1.25	15.36	18.98
(1)	320	42.58	41.75	0.83	1.25	8.96	9.36
	450	90.23	89.44	0.89	0.75	6.32	7.41
	258	31.26	29.13	2.13	1.25	32.12	32.58
(2)	350	58.69	57.55	1.14	0.75	21.56	22.69
	480	74.39	72.22	2.17	1.25	14.11	15.33
	330	19.72	18.27	1.45	1.25	10.45	11.54
(3)	440	66.81	65.14	1.67	0.80	12.54	12.68
	530	71.67	70.05	1.62	1.25	21.68	22.40
	250	55.16	54.32	0.84	1.25	14.69	15.04
(4)	660	39	37.89	1.11	1.25	18.34	18.63
	730	87.68	85.55	2.53	0.90	8.36	9.65
	190	68.39	68.10	0.29	1.25	9.82	10.14
(5)	310	39.08	37.25	1.82	1.25	18.78	19.25
	560	79.84	77.46	2.38	1.00	11.55	12.66

The NMR spectra of **1-5** exhibited the expected resonances arising from the organotin(IV) moieties and protons of N-maleoyl-L-alanine /37/. The coupling constants can yield important structural information and the magnitude of the $^{1}J[^{119}Sn\text{-}^{13}C]$ and $^{2}J[^{119}Sn\text{-}^{1}H]$ was consistent with a six-coordinated tin centre in an octahedral arrangement, thereby indicating a 1:2 metal-to-ligand stoichiometry /32, 38/. Howard's equations (1 and 2) were successfully applied for the first time in the solid-state for the estimation of C—Sn—C angle; Eq. (1) yielded 188°, 180°, 186°, 182° and 184°; and Eq. (2) gave 182°, 180°, 181°, 179° and 178° respectively for **1-5** [39].

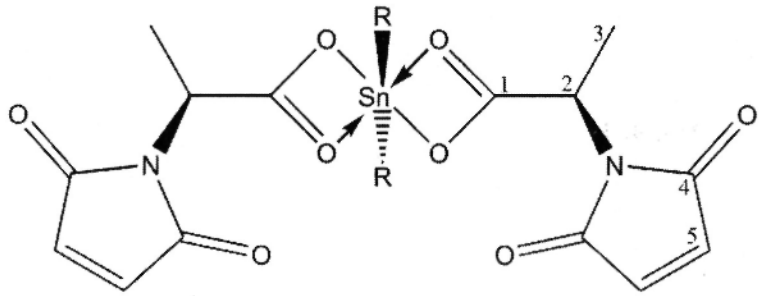
$$\angle C\text{—}Sn\text{—}C = 2.28[^{2}J(^{119}Sn\text{-}^{1}H)] - 46.4 \quad (1)$$

$$\angle C\text{—}Sn\text{—}C = 0.178[^{1}J(^{119}Sn\text{-}^{13}C)] + 14.74 \quad (2)$$

^{119}Sn NMR plays a significant role in determining the geometry around the tin atom /40/. The ^{119}Sn NMR

chemical shifts of **1-5** (ppm) were comparable with earlier reports describing octahedral geometry in the solid-state /41-44/.

All these results were comparable to the solution-state geometrical behavior of the complexes /15/, confirming the octahedral geometry (1:2 metal-to-ligand stoichiometry) in the solid-state in all the title complexes.



R: $^{6}CH_3$ **(1)** $^{6}CH_2$-$^{7}CH_3$ **(2)** $^{6}CH_2$-$^{7}CH_2$-$^{8}CH_3$ **(3)**

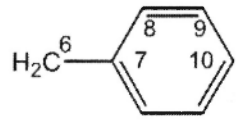
$^{6}CH_2$-$^{7}CH_2$-$^{8}CH_2$-$^{9}CH_3$ **(4)**  **(5)**

Fig. 1: Trans-octahedral geometry and numbering for ^{1}H and ^{13}C NMR.

Thermogravimetry

Thermogravimetric (TG) analysis of **1-5** revealed that decomposition of these compounds occurs with the increase of temperature. A probable degradation scheme is depicted in Fig. 2. Moreover, it was found that the weight losses observed due to thermal decomposition of **1-5** were very close to the calculated values. The slight difference in the values indicated the error, which was in the acceptable range of ±3%. From Fig. 2, we can see that in the 1st step, one of the ligands was evolved, while in the 2nd step one R group bonded to tin was detached. In the 3rd step the second ligand and the second R group were evolved simultaneously, leaving SnO as residue. The residue in **1-5** was characterized by FT-IR while the species evolved were proposed from the weight loss observed and calculated. The TG data were interpreted to calculate the kinetic parameters such as order of reaction and energies of activation of **1-5**. All the complexes showed a 1.25 order of reaction for their 1st step decomposition. However, the observed values for order of reaction of the 2nd and 3rd steps were sometimes different. The energies of activation and order of reaction at different steps were calculated by Horowitz's method /45-46/.

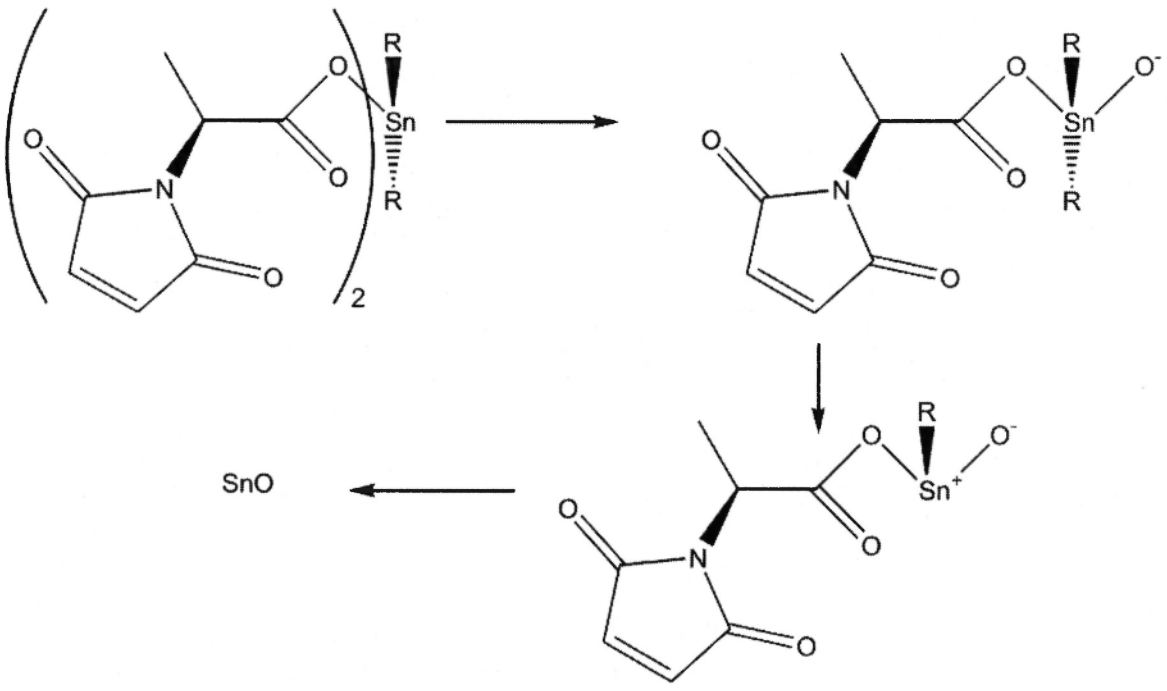


Fig. 2: Thermal degradation pattern of **1-5**.

Bioactivity

Compounds **1-5** were tested *in vitro* for their bioavailability, against five human tumoural cell lines, three insects and urease enzyme. The compounds **1-5** displayed promising *in vitro* insecticidal properties (Table 2). It is evident from the data that all the compounds displayed considerable activity at the dose of 250 μg, but when the dose was lowered, the effect gradually kept lowering and got unnoticed at a dose of 50 μg. The complexes showed significant activity, but lower than that of the standard.

Table 2

In vitro insecticidal activity of **1-5**.

Compound	Survival rate (%) @ 250, 200, 100, 50 μg		
	Pectinophora gosspiella	*Gelechiidae*	*Noctuidae*
(1)	42,31,20,08	31,29,11,03	30,20,05,00
(2)	55,40,33,19	40,22,02,00	33,05,00,00
(3)	63,54,41,40	54,31.30,12	41,30,12,04
(4)	70,61,53,51	61,40,22,04	53,41,30,20
(5)	76,66,59,55	66,21,20,10	59,44,40,22
Mortein	90,85,72,63	85,80,35,31	91,81,32,12

Table 3

In vitro inhibition doses ID_{50} (ng/ml) of compounds **1-6** against five tumoural cell lines.

Compound	H157	H1299	SK-CO-1	SW403	HT1376
1	141	152	336	776	215
2	36	41	114	256	187
3	99	100	80	71	125
4	28	39	87	69	89
5	22	96	3	15	10
6	336	214	325	258	333
Adriamycin	218	98	112	66	46
Mitomycin C	524	254	620	333	140
Vinblastine	1123	71	69	142	91

Table 3 lists the concentration that inhibited 50% of the cell growth (IDB_{50B}) for complexes (**1-5**) against H157 and H1299 lung cancers, SK-CO-1 and SW403 colon cancers and HT1376 bladder cancer of human tumour origin, along with the corresponding values of IDB_{50} for the clinically used drugs adriamycin, mitomycin C and vinblastine for comparison. All the complexes displayed significant activities in comparison to the ligand and the reference drugs, while **4** and **5** were found to be more potent.

Table 4 contains enzyme inhibition effect of **1-6**. During enzyme inhibition screening, it was observed that compounds **4** and **5** displayed potent inhibitory effect, **2-4** were moderate inhibitors while **1** and **6** displayed weak inhibition of enzyme urease. The enzyme-inhibiting ability may be attributed to coordinating nature of these complexes.

Table 4

In vitro quantitative inhibition of urease by compounds **1-6**.

Compound	$IC_{50} \pm$ Sem (Bacillus pasteurii urease)	$IC_{50} \pm$ Sem (Jack bean urease)
1	64.23 ± 0.45	71.54 ± 0.25
2	60.11 ± 0.74	65.32 ± 0.66
3	52.33 ± 0.72	48.96 ± 0.36
4	46.63 ± 0.31	40.84 ± 0.14
5	31.41 ± 0.04	29.65 ± 0.35
6	66.92 ± 0.23	89.74 ± 0.04
Thiourea (Standard)	16.07 ± 0.78	22.35 ± 0.13

Standard mean error of 3-5 assays. All the IC_{50} values are in μM

During the *in vitro* anti-tumour activity, the nature and size of R groups attached to Sn(IV), affected the *in vitro* toxicity against the tumoural cell lines used. For highlighting this statement, the average IDB_{50} data have been plotted versus the percent CH in Fig. 3. The percent CH has been defined as:

Percent CH (R) = [C_n(12.011) + H_n(1.0079) / Molecular mass of the complex] × 100

where n is the number of carbon or hydrogen atoms in R groups.

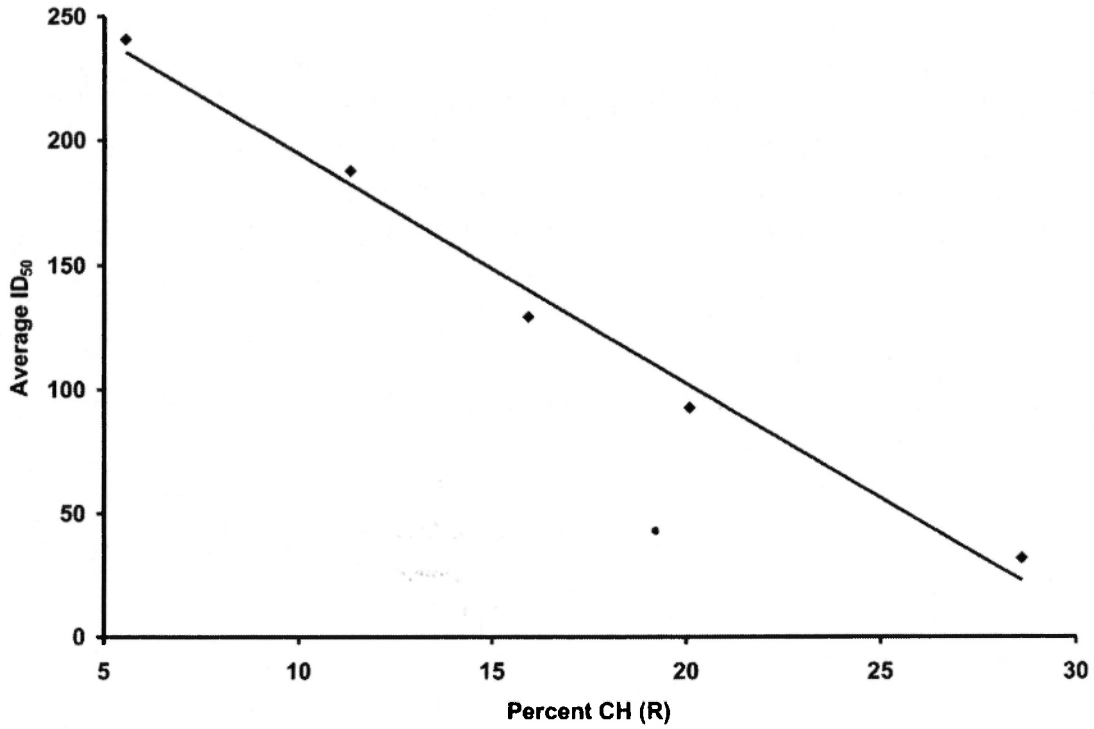


Fig. 3: Dependence of average anti-tumour activity on percent CH of R group (**1-5**).

Fig. 3 shows that the IDB_{50} decreases with the increase in percent CH almost linearly. Some deviations in case of smaller alkyl R groups have been observed, which may be attributed to variation in the conformational behavior and distribution of complexes between phases. The use of N-maleoyl-L-alanine as ligand increases the hydrophilicities of these complexes that might be responsible for such significant results. Conclusively, we can say that the bulkiness of the attached R group/percent CH values and polar character of carboxylic group of N-maleoyl-L-alanine are interlinked with each other, which enhance the polarity C—Sn and O—Sn bonds in **1-5**. A study is being carried out for the *in vivo* interactions/mechanism of action of these complexes.

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