Chapter 4

Chapter 4

4. Experimental Section

4.1 General Procedure

4.1.1 Synthetic Techniques

Hexane, petroleum ether and benzene were distilled from sodium whereas methanol was distilled after reacting with magnesium.

4.1.2 Starting Materials

The organotin starting materials, *viz.*, Me_3SnCl (Merck), Bu_3SnCl (Merck), Ph_3SnCl (Fluka), Me_2SnCl_2 (Fluka), Ph_2SnCl_2 (Aldrich), $^nBu_2SnCl_2$ (Merck) and $^tBu_2SnCl_2$ (Strem) were used as such.

The organic chemicals, *viz.*, glycine (Aldrich), 2-hydroxyacetophenone (Aldrich), salicylaldehyde (Fluka), 2-hydroxy-5-chlorobenzaldehyde (Kodak) were of reagent grade whereas 2-hydroxy-5-methylacetophenone and 2-hydroxy-3-methylacetophenone were gift samples.

4.1.3 Instrumental Methods

Elemental analyses were performed with a Perkin-Elmer 2400 series II instrument.

IR spectra in the range 4000-400 cm^{-1} were obtained on Perkin-Elmer 983 and Perkin-Elmer 1720X FT, and BOMEM DA-8 FT-IR spectrophotometers with samples investigated as KBr discs.

NMR spectra were recorded on a Bruker ACF 300 (^1H, 300.13 MHz, ^{13}C, 75.47 MHz, ^{119}Sn, 111.92 MHz) FT-NMR spectrometer. ^{119}Sn NMR spectra were also recorded, with an inverse-gated pulse delay of 2 seconds, using a JEOL GX 270 MHz FT-NMR spectrometer operating at 100.75 MHz. Chemical shifts are given in ppm and are referenced to Me_4Si for ^1H and ^{13}C, and Me_4Sn for ^{119}Sn.

^{119}Sn Mössbauer spectra of the complexes in the solid state were recorded on a Elscient-Laben spectrometer equipped with an AERE cryostat at liquid nitrogen temperature. The $Ca^{119m}SnO_3$ Mössbauer source (10 mCi; Radiochemical Centre, Amersham, UK) moved with constant acceleration and triangular waveform. The velocity calibration was made using a ^{57}Co Mössbauer source (10 mCi). An iron foil enriched to 95% in ^{57}Fe (DuPont Pharma Italia, Firenze, Italy) was used as the absorber.

Melting points were determined on a Symax melting point apparatus and were uncorrected.

4.2 Synthetic Procedures

A few representative methods for the preparation of potassium salt (L^4HK) and sodium salt (L^4HNa) are described in Sections 4.2.1 and 4.2.2, respectively. A few typical syntheses of organotin complexes of different types are described in Sections 4.2.3 -4.2.9. The organotin complexes of the same kind were prepared similarly using appropriate reactants. Their characterization, analytical and spectroscopic data are given in Chapter 2.

4.2.1 Preparation of L^4HK

A cold aqueous solution (25 ml) of KOH (2.06 g, 36.72 mmol) was mixed with a cold aqueous solution (25 ml) containing glycine (2.77 g, 36.72 mmol) and held at 15-20 °C in an ice-bath, with continuous stirring. An ethanolic solution (50 ml) of 2-hydroxy acetophenone (5.0 g, 36.72 mmol) was added dropwise. A deep-yellow colour developed almost immediately, and stirring was continued for 1h under cold conditions followed by 5h at room temperature. The solvent was removed using a rotary evaporator. The yellow mass was washed with petroleum ether and precipitated with a methanol-diethyl ether mixture. The crude product was recrystallized from methanol solution to yield L^4HK (6.09 g; 72 %), M.p. : 258-260 °C(dec).

Elemental analysis (Calcd for $C_{10}H_{10}KNO_3$, *Mw* = 231.28 g mol^{-1}):

Calcd: C, 51.9; H, 4.4; N, 6.1 %.

Found: C, 51.9; H, 4.3; N, 6.0 %.

IR (cm^{-1}): 1628 $\nu(OCO)_{asym}$, 1606 ν(C=N), 1267 ν(Ph(CO)).

The other potassium salts, *viz.*, L^2HK, L^3HK and L^6HK were prepared analogously by reacting equimolar amounts of potassium glycinate (genered *in situ*) with either 2-hydroxy acetophenone or salicylaldehyde [33, 50, 51], as appropriate, while L^1HK and L^5HK were prepared under cold conditions (-10 °C) according to the reported procedure [27]. The potassium salts of the ligands were recrystallized from methanol and obtained as bright yellow crystalline solids.

L^1HK: Yield: 0.53 g, 62 %. M. p.: 217-218 °C (dec.).

Elemental analysis (Calcd. for $C_9H_8KNO_3$, *Mw* = 217.25 g mol^{-1}):

Calcd: C, 49.76; H, 4.40; N, 6.10 %.

Found: C, 49.60; H, 4.20; N, 6.00 %.

IR (cm^{-1}): 1640 $\nu(OCO)_{asym}$, 1607 ν(C=N), 1281 ν(Ph(CO)).

L^2HK: Yield: 0.57 g, 72 %: M.p.: >250 °C (dec.).

Elemental analysis (Calcd. for $C_9ClH_7KNO_3$, *Mw* = 251.74 g mol^{-1}):

Calcd: C, 42.90; H, 2.80; N, 5.56 %.

Found: C, 42.50; H, 2.70; N, 5.50 %.

IR (cm^{-1}): 1633 $\nu(OCO)_{asym}$, 1604 ν(C=N), 1260 ν(Ph(CO)).

L^3HK: Yield: 0.59 g, 77 %. M.p.: 240 °C (dec).

Elemental analysis (Calcd. for $C_9H_7KN_2O_6$, *Mw* = 262.23 g mol^{-1}):

Calcd: C, 41.22; H, 2.60; N, 10.68 %.

Found:C, 41.02; H, 2.58; N, 10.52 %.

IR (cm^{-1}): 1646 $\nu(OCO)_{asym}$, 1606 $\nu(C = N)$, 1234 $\nu(Ph(CO))$.

L^5HK: Yield: 1.22 g, 76 %. M.p.: 205 °C (dec.).

Elemental analysis (Calcd. for $C_{11}H_{12}KNO_3$, *Mw* = 245.29 g mol^{-1}).

Calcd: C, 53.86; H, 4.93; N, 5.70 %.

Found: C, 53.70; H, 4.80; N, 5.60 %.

IR (cm^{-1}): 1629 $\nu(OCO)_{asym}$, 1603 $\nu(C=N)$, 1250 $\nu(Ph(CO))$.

L^6HK: Yield: 0.63 g, 70 %. M.p.: 259 °C (dec.).

Elemental analysis (Calcd. for $C_{11}H_{12}KNO_3$, *Mw* 245.29 g mol^{-1}):

Calcd: C, 53.86; H, 4.93; N, 5.70 %.

Found: C, 53.80; H, 4.90; N, 5.50 %.

IR(cm^{-1}): 1645 $\nu(OCO)_{asym}$, 1615 $\nu(C=N)$, 1266 $\nu(Ph(CO))$.

4.2.2 Preparation of L^4HNa

A hot aqueous solution (30 ml) of $NaHCO_3$ (3.09 g, 36.72 mmol) was added slowly to a hot aqueous solution (25 ml) containing glycine (2.75 g, 36.72 mmol) under stirring. After completion of CO_2 evolution, 2-hydroxyacetophenone (5.0 g, 36.72 mmol) in ethanol (50 ml) was added dropwise. The reaction mixture was then maintained between 40 °C and 50 °C for 2h, after which the solvent was removed using a rotary evaporator. A thick yellow mass was precipitated with methanol petroleum ether mixture. This was washed thoroughly with petroleum either and recrystallized from methanol to yield pure L^4HNa (6.26 g; 80 %). M.p.: 239-240 °C(dec.).

Elemental analysis (Calcd. for $C_{10}H_{10}NNaO_3$, *Mw* = 215.07 g mol^{-1}):

Calcd: C, 55.8; H, 4.7; N, 6.5 %.

Found: C, 55.7; H, 4.5; N, 6.5 %.

IR (cm^{-1}): 1618 $\nu(OCO)_{asym}$, 1606 $\nu(C=N)$, 1267 $\nu(Ph(C-O))$.

L^6HNa was prepared analogously by reacting 2-hydroxy-5-methylacetophenone, glycine and $NaHCO_3$. Yield: 3.27 g, 72 %). M.p.: 214 °C(dec.).

Elemental analysis (Calcd. for $C_{11}H_{12}NNaO_3$, *Mw* = 229.09 g mol^{-1}):

Calcd: C, 57.64; H, 5.28; N, 6.11 %.

Found: C, 57.50; H, 5.21; N, 6.05 %.

IR (cm^{-1}): 1640 $\nu(OCO)_{asym}$, 1603 $\nu(C=N)$, 1270 $\nu(Ph(CO))$.

4.2.3 Preparation of Ph_3SnL^1H (22)

Ph_3SnCl (1.77 g, 4.60 mmol) in benzene (25 ml) was added dropwise with continuous stirring to L^1HK (1.0 g, 4.60 mmol) in 50 ml methanol. The reaction was then refluxed for 3h and the solvent was distilled off to dryness using a rotary evaporator. The residue was washed with petroleum ether, extracted into hot chloroform and filtered. The yellow-coloured extract was distilled off (up to 50 % of the initial solvent volume) and kept at room temperature for crystallization. On the following day, the solid was isolated by filtration and recrystallized from methanol and chloroform mixture. Slow evaporation of methanol and chloroform solutions (1:1, v/v) deposited shining block-like crystals.

4.2.4 Preparation of Me_3SnL^4H (24)

Me_3SnCl (0.80 g, 4.01 mmol) in methanol (30 ml) was added dropwise with continuous stirring to a hot methanol solution (50 ml) containing either L^4HNa (0.863 g, 4.01 mmol) or L^4HK (0.927 g, 4.01 mmol). The reaction mixture was heated at reflux temperature for 3h and then the solvent was removed using a rotary evaporator. The yellow mass was washed thoroughly with hot petroleum ether, extracted into chloroform and filtered. The yellow product obtained upon concentration of the chloroform extract and was recrystallised from methanol which upon slow evaporation yielded yellow block crystals.

4.2.5 Preparation of Bu_3SnL^4H (25)

The compound was prepared by reacting Bu_3SnCl (0.64 g, 1.96 mmol) and L^4HK (0.454 g, 1.96 mmol) in anhydrous benzene (50 ml) under reflux conditions for 7h. The reaction mixture was filtered while hot and filtrate was evaporated to dryness. The yellow jelly-like residue was cooled in a freezing mixture and then petroleum ether was added dropwise to yield crude product. The crude product was washed thoroughly with petroleum ether and finally recrystallized from chloroform and petroleum ether (1/1, v/v).

4.2.6 Preparation of $^nBu_2SnL^4.OH_2$ (40)

$^nBu_2SnCl_2$ (0.50 g, 1.64 mmol) in 50 ml of methanol was added dropwise with continuous stirring to a hot methanol solution (50 ml) containing L^4HK (0.378 g, 1.64 mmol). The reaction mixture was stirred at room temperature for 3h, and the solvent was removed using a rotary evaporator. The dry mass was washed thoroughly with hot hexane, extracted into chloroform and filtered. The pale yellow product obtained upon concentration of the chloroform. This was then recrystallized from the same solvent to yield pale yellow crystals of the desired product.

4.2.7 Preparation of Ph_2SnL^4 (41)

Ph_2SnCl_2 (0.79 g, 2.32 mmol) in 30 ml of methanol was added dropwise with continuous stirring to a hot methanol solution (50 ml) containing either L^4HNa or L^4HK (0.50 g or 0.53 g, 2.32 mmol). The reaction mixture was heated at reflux temperature for 2h, and then the solvent was removed using a rotary evaporator. The dry mass was washed thoroughly with hot hexane and then extracted into chloroform. The yellow product obtained upon concentration of the chloroform extract was recrystallized from methanol to yield yellow block shaped crystals.

4.2.8 Preparation of $Vin_2SnL^4.OH_2$ (42)

Vin_2SnCl_2 (0.484 g, 2.32 mmol) was added dropwise to a stirred methanol solution (50 ml) containing L^4HNa (0.50 g, 2.32 mmol). The reaction mixture was stirred for 3h at room temperature. The volatiles were removed *in vacuo*, and the residue was

washed with hexane and then extracted into chloroform. A colourless product was obtained upon concentration of a chloroform extract and recrystallized from benzene solution to give colourless block-shaped crystals.

4.2.8 Preparation of $Ph_2SnL^4.Ph_3SnCl$ (46)

This compound was prepared by the dropwise addition of an anhydrous benzene solution (30 ml) of Ph_3SnCl (1.0 g, 2.59 mmol) to a hot benzene solution (50 ml) containing Ph_2SnL^4 (1.2 g, 2.59 mmol). The reaction mixture was refluxed for 2h, and excess solvent was removed using a rotary evaporator. The yellow gummy mass thus obtained was washed several times with hexane and recrystallized from chloroform solution which afforded nice bright yellow crystals.

4.3 Single Crystal X-ray Structural Analysis

4.3.1 General Procedure

The intensity data were collected at room temperature on a Rigaku AFC6S (for complexes **22** and **24**) and on a Rigaku AFC6R (for complexes **41, 42, 45, 46** and **47**) diffractometers employing the ω: 2θ scan technique and graphite–monochromated MoKα radiation (λ = 0.71073 Å). The data sets were corrected routinely for Lorentz and polarization effects [66] and an empirical absorption correction [67] was applied in each case. The structures of the complexes were solved by direct methods [68, 69]) and each refined by a full-matrix least-squares procedure based on F^2 (**22** and **24**) or F (**41, 42, 45, 46, 47**) [66]. Non-hydrogen atoms were refined with anisotropic displacement parameters and hydrogen atoms included in the models at their calculated positions (C-H: 0.97 Å). The diagrams were drawn with ORTEP [70] at the 50 % probability level. Crystallographic data and refinement details for the organotin complexes are given in Section 4.3.2.

4.3.2 X- ray Structural Data

Table 26: Crystallographic Parameters for Ph_3SnL^1H (22)

Molecular formula	$C_{27}H_{22}NO_3Sn$
Molecular weight	527.15
Crystal colour/ morphology	Yellow, block
Crystal dimensions (mm)	0.42 X 0.54 X 0.48
Crystal system	Monoclinic
Space group	$P2_1/n$
a (Å)	10.7790 (12)
b (Å)	13. 3111(13)
c (Å)	16.1450 (19)
α (°)	90 (0)
β (°)	91.132 (9)
γ (°)	90 (0)
V (Å^3)	2316.0 (4)
Z	4
ρ Calc.(g cm^{-3})	1.512
F(000)	1060
μ (mm^{-1})	1.131
θ limits, data (°)	2.25-27.50
No.of data measured	5591
No.of unique data	5316
No.of observed data	5316
No.of parameters	311
R (1≥2σ (1)	0.0394
R_w (1≥2σ (1)	0.0832
Maximum and minimum transmission	0.2726 and 0.2216
Residual electron density (e Å^{-3})	0.491 and –0.353
G.O.F.	1.009

Table 27 : Crystallographic Parameters for Me_3SnL^4H (24)

Molecular formula	$C_{13}H_{18}NO_3Sn$
Molecular weight	354.97
Crystal colour/ morphology	Yellow , block
Crystal dimensions (mm)	0.06 X 0.66 X 0.33
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	10.7327 (14)
b (Å)	13.010 (2)
c (Å)	10. 5513 (15)
α (°)	90 (0)
β (°)	90.458 (9)
γ (°)	90 (0)
V (Å^3)	1473. 3 (4)
Z	4
ρ Calc.(g cm^{-3})	1.600
F(000)	708
μ (mm^{-1})	1.734
θ limits, data (°)	2.49-30.0
No.of data measured	3876
No.of unique data	3744
No.of observed data	3744
No.of parameters	190
R (1≥2σ (1)	0.0310
R_w (1≥2σ (1)	0.0775
Maximum and minimum transmission	1.0000 and 0.4488
Residual electron density (e Å^{-3})	0.500 and –0.421
G.O.F.	1.061

Table 28 : Crystallographic Parameters for Ph_2SnL^4 (41)

Molecular formula	$C_{22}H_{19}NO_3Sn$
Molecular weight	464.1
Crystal colour/ morphology	Yellow, block
Crystal dimensions (mm)	0.19 X 0.32 X 0.42
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	20.280 (4)
b (Å)	20.597(5)
c (Å)	9.366 (1)
α (°)	90 (0)
β (°)	92.06 (1)
γ (°)	90 (0)
V (Å^3)	3909(1)
Z	8
ρ Calc.(g cm^{-3})	1.577
F(000)	1856
μ (mm^{-1})	1328
θ limits, data (°)	3 -27.5
No.of data measured	9628
No.of unique data	9298
No.of observed data	4767
No.of parameters	487
R (1≥3σ (1)	0.042
R_w (1≥3σ (1)	0.044
Maximum and minimum transmission	1.000 and 0.969
Residual electron density (e Å^{-3})	0.37/ - 0.44
G.O.F.	1.52

Table 29: Crystallographic Parameters for $Vin_2SnL_4.OH_2$ (42)

Molecular formula	$C_{14}H_{17}NO_4Sn$
Molecular weight	382.0
Crystal colour/ morphology	Colourless, block
Crystal dimensions (mm)	0.1 X 0.18 X 0.32
Crystal system	Triclinic
Space group	*P*1
a (Å)	9.323(4)
b (Å)	10.03(1)
c (Å)	8.756 (5)
α (°)	96.61(8)
β (°)	106.08 (5)
γ (°)	104.47(5)
V (Å^3)	746(1)
Z	2
ρ Calc.(g cm^{-3})	1.699
F(000)	380
μ (mm^{-1})	17.22
θ limits, data (°)	3 -27.5
No.of data measured	3661
No.of unique data	3452
No.of observed data	2890
No.of parameters	181
R (1≥3σ (1)	0.039
R_w (1≥3σ (1)	0.049
Maximum and minimum transmission	1.000 and 0.910
Residual electron density (e Å^{-3})	1.31/-1.52
G.O.F.	1.97

Table 30 : Crystallographic Parameters for Ph_2SnL^6 (45)

Molecular formula	$C_{23}H_{21}NO_3Sn$
Molecular weight	478.1
Crystal colour/ morphology	Yellow hexagon
Crystal dimensions (mm)	0.26X0.23X0.23
Crystal system	monoclinic
Space group	$P2_1/c$
a (Å)	10.270 (3)
b (Å)	19.496 (4)
c (Å)	10.270
α (°)	90 (0)
β (°)	106.15 (1)
γ (°)	90 (0)
V (Å^3)	1975.1(9)
Z	4
ρ Calc.(g cm^{-3})	1.608
F(000)	960
μ (mm^{-1})	13.17
θ limits, data (°)	3 -27.5
No.of data measured	5133
No.of unique data	2766
No.of observed data	1744
No.of parameters	253
R (1≥3σ (1)	0.027
R_w (1≥3σ (1)	0.026
Maximum and minimum transmission	
Residual electron density (e Å^{-3})	0.23/ -0.23
G.O.F.	1.51

Table 31 : Crystallographic parameters for Ph_2SnL^4 .Ph_3SnCl (46)

Molecular formula	$C_{40}H_{34}ClNO_3Sn_2$
Molecular weight	849.6
Crystal colour/ morphology	Pale yellow, block
Crystal dimensions (mm)	0.02 X 0.12 X 0.17
Crystal system	triclinic
Space group	*P*1
a (Å)	13.073 (2)
b (Å)	13.961 (2)
c (Å)	11.951 (2)
α (°)	92.06 (1)
β (°)	115.39 (1)
γ (°)	110.67 (1)
V (Å^3)	1743.6 (7)
Z	2
ρ Calc.(g cm^{-3})	1.618
F(000)	844
μ (mm^{-1})	15.48
θ limits, data (°)	3.0 -25.5
No.of data measured	6467
No.of unique data	6173
No.of observed data	3509
No.of parameters	424
R (1≥3σ (1)	0.038
R_w (1≥3σ (1)	0.037
Maximum and minimum transmission	1.000 and 0.963
Residual electron density (e Å^{-3})	0.50/-0.57
G.O.F.	1.35

Table 32 : Crystallographic parameters for $^{t}Bu_2SnL^4 .^{t}Bu_2SnCl_2$ (47)

Molecular formula	$C_{26}H_{34}Cl_2NO_3Sn_2$
Molecular weight	727.8928
Crystal colour/ morphology	Pale yellow, block
Crystal dimensions (mm)	0.065 X 0.19 X 0.52
Crystal system	Triclinic
Space group	*P*1
a (Å)	12.748 (2)
b (Å)	16.531 (2)
c (Å)	8.117 (1)
α (°)	96.79 (2)
β (°)	96.67(1)
γ (°)	72.45(1)
V (Å^3)	1613.7(5)
Z	2
ρ Calc.(g cm^{-3})	1.597
F(000)	780
μ (mm^{-1})	17.43
θ limits, data (°)	3.0 –27.5
No.of data measured	7790
No.of unique data	7457
No.of observed data	4018
No.of parameters	307
R (1≥3σ (1)	0.042
R_w (1≥3σ (1)	0.042
Maximum and minimum transmission	1.000 and 0.905
Residual electron density (e Å^{-3})	0.52/-0.52
G.O.F.	1.62

4.4 Experimental Protocol for Biological Work

The organotin(IV) complexes, *viz.*, L^4SnPh_2 (**41**), $L^4SnPh_2.Ph_3SnCl$ (**46**), $L^4Sn^tBu_2.^tBu_2SnCl_2$ (**47**) and L^4HNa, were dissolved in dimethyl sulfoxide (DMSO) and further diluted with DMSO in order to achieve the required working solution concentration (Table 25). The animals of the control group were treated with only DMSO solutions (0.1 ml).

Male Swiss-albino mice, aged 7 weeks and weighing about 21-23 g [maintained in the laboratory in communal cages in room under controlled temperature (27 ± 3 °C) and lighting (12h light/12h dark) conditions on standard mouse diet (NMC Oil Mills Ltd., Pune, India) and water [*ad libitum*] were used in all experiments. One hundred mice were divided randomly into five goups of 20 each.

Ehrlich ascites carcinoma (EAC) cells were maintained in 7-week-old male Swiss mice throughout intraperitoneal (i.p.) transplantation of 1 x 10^6 viable tumour cells to each mouse. On day 0, five groups of mice received tumour cells (1 x 10^6 per mouse i.p.). On day 2, the complexes to be screened, **41, 46, 47** and L^4HNa, were injected (i.p.) into four different groups of mice. One untreated group , which was received only DMSO, was kept as a control. Three animals from each group were sacrifised by cervical dislocation on the 12^{th} or 13^{th} day of cell implantation. Ascites fluid volume, packed cell volume and number of cells were then measured. The survival of drug treated mice over controls was studied in the remainder of the mice.