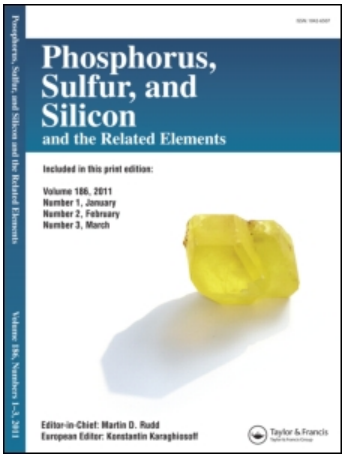

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Antitumour, Antimicrobial and Antifertility Effects of Potentially Biodynamic Sixteen to Twenty Four Membered Tetraazamacrocyclic Ligands and Their Tin(II) Complexes

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Antitumour, Antimicrobial and Antifertility Effects of Potentially Biodynamic Sixteen to Twenty Four Membered Tetraazamacrocyclic Ligands and Their Tin(II) Complexes

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Sixteen to twenty four membered tetraamide macrocyclic ligands TAML[1]-TAML[4] have been prepared by the condensation of 1, 3-diaminobutane with dicarboxylic acids malonic, succinic, glutaric or adipic in the presence of condensing reagents, dicyclohexylcarbodiimide and 4-dimethylaminopyridine. On reduction, these macrocyclic ligands provide a new series of tetraazamacrocyles with tin (II) chloride. The ligands and their complexes were characterized by elemental analyses, molecular weight determinations, infrared, ^{1}H NMR, ^{13}C NMR and X-ray spectral analysis. An octahedral geometry for these complexes has been confirmed by spectral studies. On the basis of the chemical composition the representation of the complexes as [Sn (N_4TAZML^n)Cl_2] (where N_4TAZML^n = macrocyclic ligand moiety and n = 1–4) has been established. The ligands and their complexes have been screened in vitro against a number of pathogenic fungi and bacteria to assess their growth inhibiting potential. The testicular sperm density, sperm morphology, sperm motility, density of cauda epididymis spermatozoa and fertility in mating trials and biochemical parameters of the reproductive organs have been examined and discussed. The antitumour effect of the compounds was examined on swiss mice. The results obtained clearly indicated that the compound [Sn(N_4TAZML^4)Cl_2] has effective antitumour activity.

Keywords Antitumor activity; antimicrobial activity; biodynamic; tetraamides; tetraazamacrocycles

INTRODUCTION

The importance of macrocyclic complexes is well recognized. In the past, attention has been paid to the design and synthesis of small molecular

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complexes that mimic aspects of the spectral and chemical properties of metal sites protein. The applications of macrocyclic compounds in bioinorganic chemistry and extraction of small molecules gave impetus to this endeavour.[1,2] The amide macrocyclic ligands are of interest because their metal complexes function similar to porphyrin analogues in catalysing organic oxidation ion reactions.[3] It is well known[4] that ligand cyclization has a pronounced effect on the stability of the transition metal complexes which too depends on the ring size of the macrocycles. A variety of tetraaza with different exocyclic substituents encapsulating metal ions have been reported[5] using template method. Recently, a variety of pendant arms have been introduced[6] as endocyclic substituents in the ring with a view to obtain a bigger cavity size for accommodating divalent ions in the ring system. Some workers have emphasized macrocycles with additional pendant arms to make available extra binding sites with a view to expand not only the cavity size but also the number of donor groups available to bind metal ions exocyclic or endocyclic way in the ring system. Now-a-days, interest has been focused on the synthesis of macrocyclic complexes with potential medicinal applications, cancer diagnosis[7] and in the treatment of tumor.[8] Tin complexes have a range of pharmacological applications.[9] These complexes are also used in agriculture as efficient fungicides and bactericides.[10,11]

As our programme for the development of tetraazamacrocles[12–18] and the peculiar behaviour of tin complexes in chemical and biochemical processes has led us to synthesize such type of complexes and screened them for their antitumour and antifertility activities so as to contribute in the field of bioinorganic chemistry and their clinical uses. In this context the present communication deals with the synthesis, antitumour screening and contraceptive efficacy of macrocylic complexes of tin. The aim of the antifertility activity was to assess the effect on fertility and to contribute to a better understanding of the reproductive function of male albino rats.

EXPERIMENTAL

The chemicals including dicyclohexylcarbodiimide, 4-dimethylaminopy ridine, malonic acid, succinic acid, glutaric acid and adipic acid (Fluka) were used of AR grade. 1,3-Diaminobutane and $LiAlH_4$ were used as obtained from E.Merck and $SnCl_2$ was used without further purification.

Synthesis of the Ligands (TAML[1]-TAML[4])

The reaction is carried out in 2 : 2 molar ratios. The appropriate amount of dicyclohexylcarbodiimide (1.5461 g/7.5 mmol) and catalytic amount

of 4-dimethylaminopyridine in 25 mL of dichloromethane at 0°C, put on in magnetically stirred two nacked round bottom flask. The reaction is followed by the addition of 1,3-diaminobutane in dichloromethane (25 mL) and malonic, succinic, glutaric or adipic acid (corresponding to DCHC). The resulting mixture was stirred for 10–12 hrs at 0°C. The solid product was isolated by filteration and washed several times with the same solvent and dried in vacuo. The solid products were recrystalized from benzene and dried in vacuo.

Synthesis of N_4TAZML^1-N_4TAZML^4

The reaction is carried out in 1 : 2 molar ratio. The ligands TAML^1-TAML^4 (0.98 g/3.1 mmol) were dissolved in tetrahydrofuran (30 mL) and cooled at 0°C. Lithium aluminum hydride (corresponding to ligands) in tetrahydrofuran (20 mL) was stirred for about 10 hrs in an ice bath. The reaction is followed by mixing the solution of the ligand and $LiAlH_4$. The reaction mixture was stirred under reflux for 72 hrs. After cooling it, 20 mL of 15% aqueous NaOH and then 30 mL water were added to the mixture at 0°C. The solid product was isolated by filtration and the residue repeatedly washed with hot tetrahydrofuran. The filtrate was concentrated under reduced pressure. The liquid thus dried in vacuo.

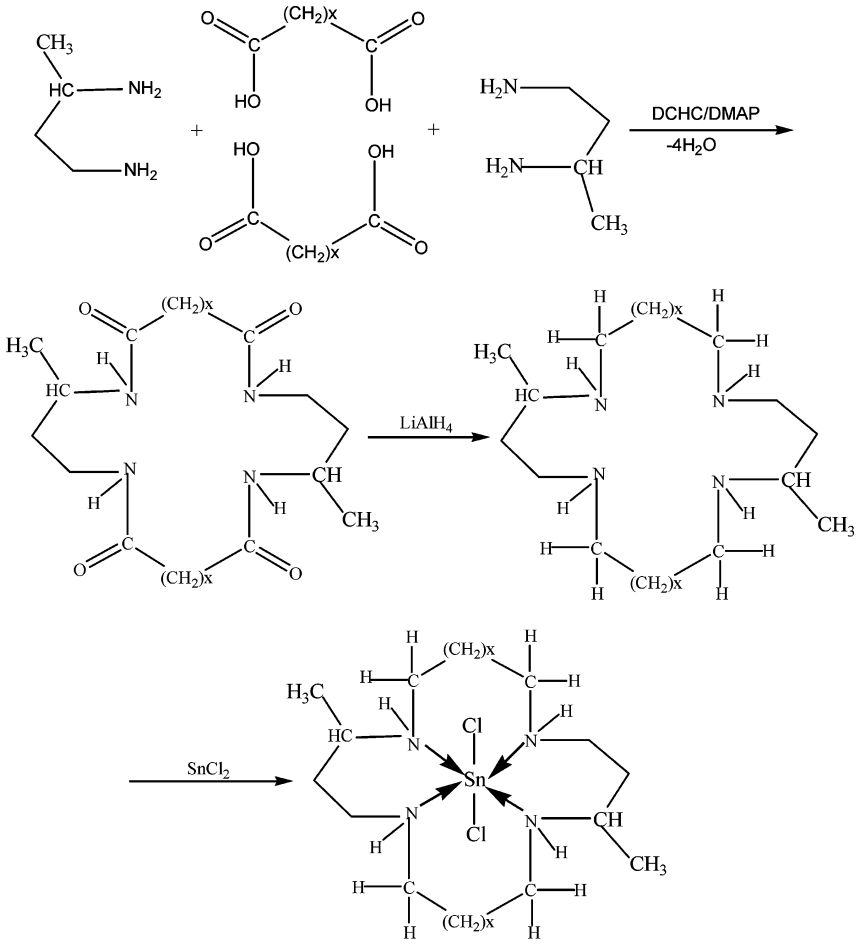
Synthesis of the Complexes

The reaction is carried out in 1 : 1 molar ratio. 0.82–0.90 g/0.32–0.39 mmol ligands, N_4TAZML^1-N_4TAZML^4 were dissolved in methanol (50 mL). The reaction is followed by the addition of $SnCl_2$solution. The resulting mixture was stirred for 12 hrs at 0°C. The solid product was obtained by filteration and washed repeatedly with the same solvent and dried in vacuo. The products were recrystallized from benzene. The purity of the ligands and their complexes were checked by TLC in DMF using pet ether as eluents.

Thus, a series of 16–24 membered tetraazamacrocyclic ligands and their complexes were derived by the condensation of dicarboxylic acids with 1,3-diaminobutane in the presence of condensing reagents DCHC and DMAP as shown in Fig. 1.

Analytical Methods and Physical Measurements

The molecular weights were determined by the Rast camphor method. Conductivity measurements in dry dimethylformamide were performed with a conductivity bridge type 305. Infrared spectra were recorded on



$TAML^1$	x= 1
$TAML^2$	x= 2
$TAML^3$	x= 3
$TAML^4$	x= 4
N_4TAZML^1	x= 1
N_4TAZML^2	x= 2
N_4TAZML^3	x= 3
N_4TAZML^4	x= 4

FIGURE 1

a Nicolet Magna FT-IR 550 spectrophotometer in Nujol Mulls. ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 using TMS as standard on a JEOL FX-90Q spectrometer. X-ray diffraction spectra of the compound was obtained on the Philips P.W. 1840 automatic diffractometer using Fe(Kα) target with Mg filter. The wavelength used was

1.9373 Å and the reflections from 5–65° was recorded. Tin was estimated gravimetrically. Carbon and hydrogen analyses were performed at the Central Drug Research Institute, Lucknow.

Bio-Chemical Procedures

Antimicrobial Activity

The antifungal activity of the ligands and their complexes with divalent tin has been evaluated by Radial Growth Method[19] using Czapek's agar medium having the composition, glucose 20 g, starch 20 g, agar-agar 20 g and distil water 1000 mL. To this medium was added requisite amount of the compound after being dissolved in dimethylformamide so as to get a certain final concentration (50, 100 and 200 ppm). The medium was then poured into the petriplates and the spores of fungi were placed on the medium with the help of an inoculum needle. These petriplates were wrapped in polythene bags containing a few drops of alcohol and were placed in an incubator at 30 ± 1°C. The controls were also run and three replicates were used in each case. The linear growth of the fungus was obtained by measuring the fungal colony diameter after four days. The percentage inhibition was calculated as 100(C-T)/C, where C and T are the diameters of the fungus colony in the control and test plates, respectively.

The organisms used in these investigations included *Scelerotium rolfsii* and *Macrophomina phaseolina*.

The activity against bacteria was evaluated by the Inhibition Zone Technique.[20] The nutrient agar medium having the composition peptone 5 g, beef extract 5 g, NaCl 5 g, agar-agar 20 g and distil water 1000 mL was pipeted into the petridish. When it solidified, 5 ml of warm seeded agar was applied. The seeded agar was prepared by cooling the molten agar to 40°C and then added the amount of bacterial suspension. The compounds were dissolved in dimethyl formamide in 500 and 1000 ppm concentrations. Paper discs of Whatman No. 1 filter paper measuring diameter of 5 mm were soaked in these solutions of varied concentrations. The discs were dried and placed on the medium previously seeded with organisms in petriplates at suitable distance. The petri plates were stored in an incubator at 28 ± 2°C for 24 hours. The zone of inhibition thus formed around each disc containing the test compounds was measured accurately in mm. The organisms used in these investigations included *Pseudomonas cepacicala* (-) and *Klebsella aerogenous* (-).

Antifertility Activity

The sprague dawley albino rats (*Rattus norvegicus*) obtained from Hamdard University, New Delhi were housed in plastic cages at room temperature (20° ± 5°C) and uniform light. They were fed on standard

laboratory chow (Ashirward Food Industries Ltd., Chandigarh, India) and fresh water *ad libitum*. The LD_{50} is statistically derived single dose of a substance that can be expected to cause death in 50% of the animals. In a prohibited analysis method of LD_{50}, the selected dose levels should bracket the expected LD_{50} value with at least one dose level higher than the expected LD_{50} but not causing 100% mortality and one dose level below the expected LD_{50} but not causing 0% mortality. Toxicity of the complexes was determined by calculating the LD_{50} values. Symptoms of poisoning and mortality were observed and results of toxicity were analyzed for determination of LD_{50} values of the complexes. On the basis of LD_{50} values the present (only single) dose of the compounds were decided for the experiment (Table I).

Proven fertile male rats (weighing 200–240 gm) were divided into six groups of five animals each. The control group A received the vehicle (olive oil) only. Animals of groups B and C received adipic acid and 1,3-diaminobutane respectively (20 mg/kg.b.wt. orally for 60 days) whereas the animals of groups D, E and F received $TAML^4$, N_4TAZML^4 and $[Sn(N_4TAZML^4)Cl_2]$, respectively (25 mg/kg. b.wt/orally) for a period of 60 days. The male rats were kept on fertility test after 60 days of treatment. They were cohabited with proesterous females in the ratio 1:4 for fertility test. Successful mating was confirmed by the presence of sperm in the vaginal smears. Females were separated and resultant pregnancies were noted when dam gave birth.

The animals were weighed and autopsied under light ether anesthesia. Sperm motility in cauda epididymis and density of testicular and cauda epididymis in suspended sperm were calculated. Reproductive organs were excised, blotted free of blood, and weighed. The testes were frozen for biochemical estimations. Total protein, glycogen, total cholesterol, sialic acid, acid and alkaline phosphatase activities were estimated by using the standard

TABLE I LD_{50} Value for Antifertility Activity for tin(II) Complexes

S. No.	Dose (mg/day/rat)	Male albino rats taken	Death
1.	250	30	30
2.	200	30	25
3.	175	30	20
4.	150	30	18
5.	100*	30	15

*LD_{50} = 100 mg/day/rat.

laboratory techniques. Student's 't' test[21] was applied in comparing the means.

Antitumor Activity

Thirty swiss mice were used for the experiments. These were divided into six groups containing five animals each. The first group served as 2% SCMC, second group N_4TAZML^3, third group N_4TAZML^3, fourth group $[Sn(N_4TAZM\ L^3)Cl_2]$, fifth group $[Sn(N_4TAZML^4)Cl_2]$, and sixth group as standard. They were selected from an inbred colony maintained under uncontrolled conditions of light, temperature ($23 \pm 3^{\circ}C$) and humidity ($50 \pm 5\%$). Mice were housed in propylene cage. The animals were fed on autoclaved mice feed and water. Six to eight weeks old female mice, having weights 25 ± 5 g were used.The LD_{50} values for this experiment is given in Table II. The test compounds were administered intraperitonially at a dose of 50 mg/kg weight in the form of suspension using 2% sodium carboxy methyl cellulose as suspending agent and the experimental dose was selected as half of the LD_{50} dose calculated by using Ghosh[22] method . All the experiments were performed according to the protocols and recommendations of the Institutional Animal Ethics Committee. The Manu Whitney U Test was applied for the median survival time. One way analysis of variance with Post hoc Sceffe's test was applied to all other parameters.

Brine Shrimp Bioassay[23] was carried out as a preliminary test to screen the drugs for antitumour activity. LC_{50} and 95% confidence intervals were determined from the 24h counts using the prohibit analysis method described by Finney[24] and compounds were selected for *in vivo* animal studies. The antitumour activity was determined by the survival time assay method[25] and Ehrlich ascitic carcinoma cells (EAC) were used. EAC was obtained from the Rajeev Gandhi Cancer Institute New Delhi, India and was propagated by serial transplantation

TABLE II LD_{50} Value for Antitumour Activity for tin(II) Complexes

S. No.	Dose (mg/kg)	Mice taken	Death
1.	200	40	40
2.	175	40	40
3.	150	40	38
4.	100	40	35
5	75	40	30
6	50*	40	20

*LD_{50} = 50 mg/kg /mice.

in Swiss mice in animal house. An intraperitonial infection of 2×10^6 tumour cells was given to each mice at weekly intervals.

Development of EAC. The mice were used 12 days after tumour transplantation. The ascitic fluid was drawn using an 18 gauge needle into a sterile syringe. An aliquot of tumour fluid was tested for microbial contamination. Tumour viability was determined by tryptan blue exclusion test and cells were counted using haemocytometer or cell counter. The ascitic fluid was suitably diluted in Baline to get a concentration of 10^6 cells / 200 μl of tumour cell suspension. This was infected intraperitonially to obtain ascitic tumour.

EAC Response. The mice were weighed on the day of tumour inoculation. The drug treatment was started 24 hours later and administered daily for seven days. The mice were weighed on every third day. Tumour response was assessed on the basis of median survival time (MST) and percent treated / control. Increased life span was calculated[26] by ILS = [(MST of treated / MST of control) -1] $\times$ 100. Percent treated = [MST of treated group / MST of control group] $\times$ 100. An enhancement of life span by 125 or more over that of control was considered as effective antitumour response.[27]

RESULT AND DISCUSSION

All these complexes are coloured solids, stable to air and non-hygroscopic. All the complexes are springly soluble in common non-polar organic solvents**.** The conductivity measured for 10^{-3} M solution in anhydrous DMF are in the range 20–27 ohm^{-1} cm^2 mol^{-1} showing them to be non-electrolytes. Elemental analyses agree well with the stoichiometry and chemical formula of the compound $[Sn(N_4TAZML^n)Cl_2]$, The physical properties and analytical data of the complexes are given in Table III.

Spectral Aspects

The preliminary identification of the macrocyclic ligands and their complexes have been obtained from their infrared spectra. The first feature of all the complexes that attracts attention is the absence of NH_2 stretching vibrations of the amine and $-OH$ groups of the dicarboxylic acids implying their involvement in the formulation of tetraamidemacrocycles. A single sharp band observed for the ligands $TAML^1$-$TAML^4$ in the region 3268–3225 cm^{-1} may be assigned to ν(N-H) of amide group. The amide I, amide II, amide III and amide IV groups are present at 1625–1695, 1553–1589, 1256–1285 and 629–675 cm^{-1},

TABLE III Physical Properties and Analytical Data of the Macrocyclic Ligands and Their Corresponding Tin(II) Complexes

Compound	M.P. (°C) and Colour	Analysis % Found (Calcd.) C	H	N	Cl	Sn	Mol. Weight Found (Calcd.)
TAML[1]	181 Off white	53.62 (53.82)	7.62 (7.74)	17.08 (17.93)	—	—	289 (312.43)
TAML[2]	162 Off white	56.25 (56.45)	8.18 (8.29)	15.74 (16.46)	—	—	314 (340.43)
TAML[3]	198 Off white	58.57 (58.68)	8.55 (8.75)	14.50 (15.21)	—	—	339 (368.43)
TAML[4]	209 Off white	60.50 (60.60)	9.02 (9.15)	13.32 (14.13)	—	—	364 (396.43)
N_4TAZML[1]	195 Light grey	65.36 (65.58	12.38 (12.58)	21.07 (21.85)	—	—	232 (256.43)
N_4TAZML[2]	217 Light grey	68.01 (68.12)	12.56 (12.76)	18.86 (19.70)	—	—	254 (284.43)
N_4TAZML[3]	183 Grey	69.00 (69.20)	12.80 (12.90)	17.18 (17.93)	—	—	286 (312.43)
N_4TAZML[4]	227 Grey	70.50 (70.65)	13.01 (13.03)	15.67 (16.46)	—	—	311 (340.43)
[Sn(N_4TAZML[1])Cl_2]	232 Creem	37.50 (37.70)	7.02 (7.23)	11.81 (12.56)	15.25 (15.90)	13.95 (14.51)	420 (346.02)
[Sn(N_4TAZML[2])Cl_2]	219 Creem	40.44 (40.54)	7.54 (7.65)	11.11 (11.82)	14.31 (14.96)	12.97 (13.51)	444 (374.03)
[Sn(N_4TAZML[3])Cl_2]	235 Creem	42.92 (43.06)	7.83 (8.03)	10.38 (11.16)	13.51 (14.12)	12.96 (13.51)	481 (502.03)
[Sn(N_4TAZML[4])Cl_2]	238 Creem	45.10 (45.32)	8.11 (8.37)	19.72 (19.32)	12.74 (13.38)	12.13 (12.64)	498 (530.03)

respectively.[28] It provide a strong evidence for the presence of a closed cyclic product. Strong and sharp absorption bands appeared in the regions 2824–3058 and 1410–1478 cm^{-1} in all the complexes are assigned to C—H stretching and C-H bending vibrational modes, respectively.[29] It has been noticed that tetraazamacrocycles N_4TAZML[1]-N_4TAZML[4] do not show amide bands corresponding to tetraamide macrocycles. However, a slight negative shift in the NH stretching vibration has been observed. All other bands do not show any appreciable change.

In the spectra of macrocyclic complexes[Sn(N_4TAZML[1])Cl_2]-[Sn(N_4TAZML[4])Cl_2] as compared to their tetraazamacrocycles, the slight negative shift in the ν(N—H) band which appeared in the region 3200–3220 cm^{-1} was noticed. It is ascribed to the coordinated N-H stretching vibration. This is further substantiated by the fact that all the complexes show a medium intensity band in the region 430–448 cm^{-1} which is attributed to Sn—N stretching vibrations.[30] The

TABLE IV IR Spectral Data (in cm^{-1}) of the Ligands and Their Corresponding Tin(II) Complexes

		Amides				CH		
Compound	ν(N—H)	I	II	III	IV	Stretching	Bending	ν(N—H)
$TAML^1$	3280	1653	1549	1256	664	2955	1415	—
$TAML^2$	3275	1679	1533	1262	675	2910	1432	—
$TAML^3$	3278	1625	1555	1277	638	2828	1446	—
$TAML^4$	3286	1695	1589	1285	629	2827	1459	—
N_4TAZML^1	3264	—	—	—	—	3019	1419	—
N_4TAZML^2	3257	—	—	—	—	3037	1451	—
N_4TAZML^3	3242	—	—	—	—	3022	1470	—
N_4TAZML^4	3250	—	—	—	—	3029	1468	—
$[Sn(N_4TAZML^1)Cl_2]$	3233					2824	1410	430
$[Sn(N_4TAZML^2)Cl_2]$	3227	—	—	—	—	3042	1457	446
$[Sn(N_4TAZML^3)Cl_2]$	3211	—	—	—	—	3049	1478	448
$[Sn(N_4TAZML^4)Cl_2]$	3223	—	—	—	—	3054	1461	433

bands around 365–375 cm^{-1} is assignable to Sn-Cl vibrations in the complexes[31]. The infrared spectral data of the ligands and their complexes have been recorded in Table IV.

The mode of bonding and the geometry of the tetraazamacrocycles were further confirmed by recording the ^1H NMR spectra of the starting materials and their respective ligands in DMSO-d_6 using tetramethylsilane (TMS) as the internal standard. The chemical shift values (δ, ppm) are given in Table V. In the spectra of free diamines and dicarboxylic acids, proton resonance signals due to $-NH_2$ and $-OH$ groups were observed, which disalppear completely in the spectra of the ligands indicating the condensation and formation of proposed frame work. In the spectra of the ligands $TAML^1$ - $TAML^4$ a broad signal observed at δ 8.04–8.15 ppm due to amide (CO—NH) protons.[32] A multiplet arising due to methylene protons (N—CH_2) appear in the region δ 3.40–3.50 ppm in all the ligands. A multiplet observed at δ 1.96–2.08 ppm, ascribed to the middle methylene protons (C—CH_2—C) of 1,3-diaminobutane moiety. A singlet appearing in the region δ 2.86–2.90 and 3.05–3.07 ppm assignable to methylene protons of malonic and succinic acid moiety respectively. However, doublets appeared in the regions δ 3.17–3.19 and 3.22–3.27 ppm attributed to methylene protons of glutaric and adipic acid moiety.

The conclusion drawn from the IR and ^1H NMR spectra are parallel with the carbon-13 spectral data regarding the authenticity of the proposed skelton. The shift of the carbons (Table 6)

TABLE V ^{1}H NMR Spectra Data (δ, ppm) of Macrocyclic Ligands and Their Corresponding Tin(II) Complexes.

Compound	CO—NH	N—CH_2	C—CH_2-C	CO—CH_2—CO/ $CO(CH_2)_2$—CO	$CO(CH_2)_3$—CO/ $CO(CH_2)_4$—CO
TAML[1]	8.04	3.40	1.96	2.86	—
TAML[2]	8.15	3.42	2.08	3.05	—
TAML[3]	8.10	3.50	2.04	—	3.17
TAML[4]	8.07	3.33	1.97	—	3.25
N_4TAZML[1]	—	3.48	1.99	2.90	—
N_4TAZML[2]	—	3.41	1.96	3.07	—
N_4TAZML[3]	—	3.44	2.02	—	3.18
N_4TAZML[4]	—	3.50	2.03	—	3.27
[Sn(N_4TAZML[1])Cl_2]	—	3.41	2.08	2.88	—
[Sn(N_4TAZML[2])Cl_2]	—	3.40	1.98	3.06	—
[Sn(N_4TAZML[3])Cl_2]	—	3.47	2.02	—	3.19
[Sn(N_4TAZML[4])Cl_2]	—	3.52	2.06	—	3.22

attached to nitrogen further supported the proposed coordination in the ligands.

The ^{119}Sn NMR spectra of the complexes give signals at -δ 550–563 ppm, indicating coordination number six in these complexes around the tin atom.[33]

TABLE VI ^{13}C NMR Spectra Data (δ, ppm) of Macrocyclic Ligands and Their Corresponding Tin (II) Complexes.

Compound	> C = O	>N—CH_2	—C—CH_2—C	C_α	C_β
TAML[1]	176.26	40.76	36.73	33.91	—
TAML[2]	169.39	43.02	36.70	33.62	—
TAML[3]	168.30	40.74	35.47	32.24	26.63
TAML[4]	178.20	45.20	36.49	33.38	27.98
N_4TAZML[1]	—	40.88	36.79	33.29	—
N_4TAZML[2]	—	44.42	37.19	32.98	—
N_4TAZML[3]	—	32.40	35.20	32.66	28.02
N_4TAZML[4]	—	40.98	36.96	32.96	26.10
[Sn(N_4TAZML[1])Cl_2]	—	42.63	35.20	32.54	—
[Sn(N_4TAZML[2])Cl_2]	—	44.10	35.13	32.36	—
[Sn(N_4TAZML[3])Cl_2]	—	42.88	35.55	32.66	28.02
[Sn(N_4TAZML[4])Cl_2]	—	43.90	35.41	31.96	26.10

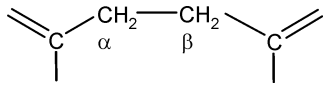


TABLE VII X-ray Powder Diffraction Data for the Compound [$Sn(N_4TAZML^4)Cl_2$]

S. No.	2θ (Obs)	2θ (Calcd)	delta	h	k	l	d-specing (obs) Å
1.	15.36	15.35	0.00	2	2	0	7.25
2.	16.91	16.89	0.02	3	0	1	6.590
3.	22.62	22.59	0.03	5	1	0	4.940
4.	25.85	25.85	0.00	4	3	0	4.330
5.	27.47	27.47	0.00	5	2	1	4.080
6.	30.78	30.76	0.02	3	4	1	3.650
7.	31.95	31.91	0.04	3	3	2	3.520
8.	37.94	37.91	0.03	7	3	1	2.980
9.	42.21	42.22	0.00	4	3	3	2.690
10.	44.62	44.65	0.00	10	1	0	2.550
11.	46.38	46.38	0.00	2	7	0	2.460
12	48.25	48.18	0.07	8	5	0	2.370

Refined Values of a = 25.775; b = 17.542; c = 10.297 $\alpha = \beta = \gamma = 90^\circ$ (Orthorhombic System).

In order to ascertain the crystal lattice of these compounds X-ray diffraction of the compound [$Sn(N_4TAZML^4)Cl_2$] has been recorded. The observed interplanar spacing values ('d' in Å) have been measured from the diffractogram of the compound and the miller indices h, k and l have been assigned to each d value and 2θ angles are reported in Table VII. The results show that the compound, belongs to 'orthorhombic' crystal system having unit cell parameters as a = 25.775, b = 17.542, c = 10.247 $\alpha = \beta = \gamma = 90^\circ$, respectively, max dev. of $2\theta = 0.9$

On the basis of the said spectral analyses, it is clear that the ligands are acting as tetradentate chelating agents having four coordination sites. Also since the anions Cl remain bonded with tin atom, a hexacoordinated environment for tin has been proposed.

Antimicrobial Activity

The results of biological activity have been compared with the conventional fungicide *Bavistin* and the conventional bactericide *Streptomycin* used as standards. The results achieved out of these studies have been enlisted in Tables VIII and IX in which the antifungal activity indicated that the metal chelates are more active than ligands and complexes showed similar type of behavior as indicated in antifungal activity. The chelation theory[34]accounts for the increased activity of the metal complexes. The chelation reduces the polarity of the metal atom

TABLE VIII Fungicidal Screening Data (% Inhibition after 96 h) of Macrocyclic Ligands and Their Corresponding tin(II) Complexes

	Scelerotium rolfsii			*Macrophomia phaseolina*		
Compounds	50 ppm	100 ppm	200 ppm	50 ppm	100 ppm	200 ppm
$TAML^1$	38	61	78	29	51	71
$TAML^2$	46	62	81	34	—	60
$TAML^3$	31	57	70	49	70	83
$TAML^4$	—	—	—	47	68	90
N_4TAZML^1	—	—	60.7	32	54	76
N_4TAZML^2	58	66	—	—	55	76
N_4TAZML^3	72	79	86	74	81	85
N_4TAZML^4	78	82	90	58	65	79
$[Sn(N_4TAZML^1)Cl_2]$	80	86	92	36	55	—
$[Sn(N_4TAZML^2)Cl_2]$	76	90	91	53	76	92
$[Sn(N_4TAZML^3)Cl_2]$	82	94	—	82	92	98
$[Sn(N_4TAZML^4)Cl_2]$	83	96	95	71	77	—
Bavistin	87	100	100	87	100	100

mainly because of partial sharing of its positive charge with the donor groups and possible π electron delocalisation within a whole chelating ring. The chelation increases the lipophilic nature of the central atom which subsequently favours its permeation through the lipid layer of the cell membrane.

TABLE IX Bactericidal Screening Data (% Inhibition after 24 h) of the Macrocyclic Ligands and Their Corresponding tin(II) Complexes

	Staphylococcus aureus (+)		*Klebsella aerogenous*(−)	
Compound	500 ppm	1000 ppm	500 ppm	1000 ppm
Streptomycin	24	35	36	58
$TAML^1$	—	32	35	61
$TAML^2$	20	29	—	59
$TAML^3$	22	35	39	60.8
$TAML^4$	—	—	35	64.2
N_4TAZML^1	42	53	59	84
N_4TAZML^2	36	47	42	57
N_4TAZML^3	59	83	84	85
N_4TAZML^4	60	68	—	—
$[Sn(N_4TAZML^1)Cl_2]$	47	59	65	90
$[Sn(N_4TAZML^2)Cl_2]$	53	65	75	92
$[Sn(N_4TAZML^3)Cl_2]$	71	94	92	100
$[Sn(N_4TAZML^4)Cl_2]$	88	12	25	42

TABLE X Effects of Starting Materials, Macrocyclic Ligand and its tin(II) Complexes on Body and Reproductive Organ Weights of Male Rats

		Body weight (g)			Organ weight (mg)		
Group	Treatment	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate
A	Control	225 ± 22.5	238 ± 21.5	1390 ± 30.0	450.0 ± 18.9	485.0 ± 22.2	478.0 ± 21.8
B	Adipic acid	220 ± 18.5	232 ± 15.7^{ns}	1180 ± 30.5^{a}	390 ± 15.0^{a}	405 ± 18.9^{b}	408 ± 16.5^{a}
C	1,3-Diaminobutane	222 ± 14.0	230 ± 18.6^{ns}	1190 ± 28.7^{b}	380 ± 14.5^{b}	410 ± 9.8^{b}	413 ± 18.9^{a}
D	$TAML^4$	228.6 ± 13.4	240 ± 12.5^{ns}	740 ± 20.5^{b}	225 ± 15.6^{b}	225 ± 13.5^{b}	232 ± 14.5^{b}
E	N_4TAZML^4	215 ± 18.0	230 ± 16.5^{ns}	910 ± 17.8^{b}	330 ± 18.4^{b}	312 ± 14.3^{b}	285 ± 14.1^{b}
F	$[Sn(N_4TAZML^4)Cl_2]$	210 ± 13.4	227 ± 15.4^{ns}	820 ± 27.6^{b}	310 ± 15.3^{b}	290 ± 15.2^{b}	315 ± 11.5^{b}

(Mean $\pm$ SEM of 5 animals)

$a = p \leq 0.01$; $b = P \leq 0.001$; ns = Non significant

Groups B and C compared with group A ; Groups B and C compared with group D,E and F.

TABLE XI Altered Sperm Dynamics of the Starting Materials, Macrocyclic Ligand and its Corresponding tin(II) Complex on Treated Rats

Group	Treatment	Sperm motility (Cauda epididymis) (%)	Sperm density (million/cm^3)		Fertility tests (%)
			Testes	Epididymis	
A	Control	86.0 ± 4.9	5.10 ± 0.30	97.9 ± 4.5	100 (+)
B	Adipic acid	50.0 ± 4.3^b	3.9 ± 0.15^b	45.5 ± 3.9^b	50 (−)
C	1,3-Diaminobutane	52.0 ± 5.4^b	3.75 ± 0.17^b	43.5 ± 3.1^b	55 (−)
D	$TAML^4$	28.5 ± 6.5^b	2.1 ± 0.11^b	20.5 ± 2.8^b	90 (−)
E	N_4TAZML^4	36.1 ± 5.8^b	3.1 ± 0.12^b	35.5 ± 2.9^b	83 (−)
F	$[Sn(N_4TAZML^4)Cl_2]$	32.5 ± 7.1^b	2.8 ± 0.13^b	30.5 ± 3.4^b	98 (−)

(Mean ± SEM of 5 animals) a = $p \leq 0.01$ b = $P \leq 0.001$ ns = Non significant
Groups B and C compared with group A ; Groups B and C compared with group D,E and F.

Antifertility Activity

No significant change in the body weights of rats were observed after treatment with the starting materials, ligands and their tin complexes. However, significant decrease in the weight of testes, epididymis, ventral prostate and seminal vesicle were observed in the treated animals (Table X).

Sperm Motility and Density

Oral administration of the starting materials and tin complexes resulted in a significant decline in the sperm motility in cauda epididymis and sperm density in testes and cauda epididymis (Table XI).

Fertility

The sluggish motile sperm were unable to fertile normal cyclic females. The test was 50 to 98 percent negative in treated animals. (Table XI)

Testicular Biochemistry

A significant reduction in protein and sialic contents of testes were observed in the starting materials and their complexes treated rats, whereas the testicular cholesterol, glycogen, acid and alkaline phosphatase contents were increased significantly (Table XII).

The present study showed that oral administration of the starting materials, ligands and tin complexes for 60 days in male rats resulted in a significant reduction in the weights of testes and other reproductive organs. The reduction in the weight of testes reflects regressive changes in the semiinferous tubules.

TABLE XII Effects of Starting Materials, Macrocyclic Ligand and its Corresponding tin(II) Complex on Total Glycogen, Protein, Cholesterol, Sialic Acid and Phosphatase Contents of Various Reproduction Organs of Male Rats

Group	Treatment	Glycogen (mg/g wet-wt.)	Total protein (mg/g wet-wt.)	Total cholesterol (mg/g wet-wt.)	Sialic acid (mg/g wet-wt.)	Phosphatase (mg/ip/g/hr)	
						Acid	Alkaline
A	Control	3.95 ± 0.19	228.8 ± 20.3	5.8 ± 0.35	5.7 ± 0.34	3.21 ± 0.15	11.39 ± 0.65
B	Adipic acid	4.40 ± 0.20^a	182.5 ± 11.0^a	6.2 ± 0.11^a	4.9 ± 0.45^b	4.2 ± 0.16^a	13.90 ± 0.65^a
C	1,3-Diaminobutane	4.45 ± 0.30^a	188.5 ± 12.5^a	6.4 ± 1.5^a	4.7 ± 0.38^b	4.1 ± 0.14^a	14.2 ± 0.75^b
D	$TAML^4$	6.14 ± 0.14^b	130.5 ± 17.5^b	8.1 ± 0.251^a	2.5 ± 0.15^b	6.2 ± 0.15^b	15.0 ± 0.56^b
E	N_4TAZML^4	5.9 ± 0.11^b	165.3 ± 8.7^b	7.2 ± 0.31^b	3.1 ± 0.45^b	5.8 ± 0.16^b	13.6 ± 0.48^b
F	$[Sn(N_4TAZML^4)Cl_2]$	5.80 ± 0.18^b	145.5 ± 12.3^b	7.8 ± 0.35^b	3.5 ± 0.17^b	5.6 ± 0.12^b	13.4 ± 0.45^b

(Mean $\pm$ SEM of 5 animals); a = $p \leq 0.01$; b = $P \leq 0.001$ and ns = Non significant.
Groups B and C compared with group A; Groups B and C compared with group D, E and F.

TABLE XIII EAC Response Data of tin (II) Complexes for *in vivo* Animal Studies

Group	Compound	Dose (mg/kg)	MST (days)*	ILS**	Percent treated/ control
Group I	2% SCMC	—	16.6 ± 1.69	—	—
Group II	N_4MacL^3	30	16.9 ± 1.07	1.8	101.8
Group III	N_4MacL^4	30	17.2 ± 1.96	3.6	103
Group IV	$[Sn(N_4MacL^3)Cl_2]$	30	19.5 ± 1.02	17.47	117.5
Group V	$[Sn(N_4MacL^4)Cl_2]$	30	20.7 ± 1.02	24.68	124.6
Group VI	Cisplatin (Standard)	3.5	24	60	150

Abbrivations : MST = Mean Survival Time, ILS = Increased Life Span

*Percent treated = MST of treated group/MST of control group × 100.

**ILS = [(MST of treated/MST of control) -1] × 100.

A redcution in the number of germinal cells leads to the reduction in the weight of testes.[35] Reduction in the weight of accessory sex organs may be due to the reduced androgen availability by these compounds.[36] The reduction in the sperm density in the cauda epididymis may be due to the alteration in the androgen metabolism[37] Suppression of gonadotropins might have caused decrease in the sperm density in the testes.[38] Further, reduction in fertility by these compounds may be due to lack of forward progression and reduction in the density of the spermatozoa and altered biochemical million of cauda epididymis. These complexes also induces biochemical changes in reproductive tract. A significant reduction in the total protein and sialic acid contents of testes after treatment indicated reduced androgen supply to those organs and decrease in the number of spermatozoa in lumen.[39] The increase in the cholesterol contents of testes may be due to non-utilization of substances for androgen biosynthesis by fewer leyding cells present or due to damage.[40]

Further, an increase in the acid and alkaline phosphatase activity in testes of the starting materials, ligands and their tin complexeS treated rats suggested an impairment of functional integrity of testes.[31] In conclusion, our results suggested that addition of ligands to the starting materials enhance their activity. The ligand $TAML^4$ is more effective then the ligand N_4TAZML^4. Furthermore. The tin complex is more effective as compared to both the ligands. It has been concluded that the complex was found to be effective fertility inhibitor in male rats and its activity due to the synergistic action of the Sn(II) moiety.[41]

Antitumour Activity

Some of the selected compounds were screened for their preliminary antitumour studies (Brine Shrimp Analysis). Table 8 indicates that all the compounds increase the life span of the tumour bearing mice. Increase life span of the compounds N_4TAZML^3, N_4TAZML^4, $[Sn(N_4TAZML^3)Cl_2]$ and $[Sn(N_4TAZML^4)Cl_2]$ are 1.8, 3.6, 17.5 and 24.7, respectively. However, the percent treated for $[Sn(N_4TAZML^3)Cl_2]$ was 117.5 and the value for$[Sn(N_4TAZML^4)Cl_2]$ was 124.6 which are more closer to the significant value 128 that is why they have effective anti tumour response.

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