# FERTILITY REGULATION IN MALE RATS BY IMPLEMENTED ORGANOTIN MACROCYCLIC COMPLEXES: SYNTHESIS, SPECTROSCOPIC ELUCIDATION AND PHARMACOLOGICAL EVALUATION

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**ABSTRACT**

*The coordination and organometallic chemistry of metal nitrogen and metal-sulfur bonded compounds have come to occupy a prominent position in the research due to their economical importance in the field of agricultural, medicinal and industrial chemistry. Although $N_4$-tetraamide derivatives of transition metal ions have been synthesized and some of their structures, interaction with main group metal ions and the formation of their coordination complexes are subjects of current interest. This communication deals with a description of synthetic procedure and structural characterization on the bais of analytical and spectroscopic techniques of a new class of coordination compounds of organotin(II) with $N_4$-tetraamide moiety derived by the dicarboxylic acids and 1,4-diaminobutane. Attempts have been made to assess the comparative growth-inhibiting potential of the synthesized complexes against a variety of fungal and bacterial strains. The results demonstrate that the concentrations reached levels sufficient to inhibit and kill the pathogens. The results of the biological studies have also been compred with a conventional standards, Bavistin and Streptomycin, taken for antifungal and antibacteral activities, respectively. The emphasis has been given in vivo on male rats by performing bio-chemistry and fertility test. The results of these findings have been discussed in detail.*

***Keywords:*** *Organotin, Macrocyclic complexes, biochemical perspective, antifertility activity and pharmacological evaliation.*

## INTRODUCTION

The fast moving and expanding development in the chemistry of coordination compounds as out lined by individual scientific backgrounds, individual interest and personal idiosyncrasies has been released due to their applicability in diverse areas of current interest mainly in agriculture and medicine.[1,2] Recently, it has been shown that the involvement of the periodic elements with organic moieties having nitrogen and sulphur atoms[3] plays a crucial role in designing a potential molecule of specific use. The field of macrocyclic chemistry of the metals is developing very fast because of its variety of applications[4] and importance in the areas of coordination chemistry.[5] There has been a spectacular growth in the interest in metal complexes with the tetraazamacrocyclic ligands followed by an extensive work on the metal controlled template synthesis of the macrocyclic species.[6] The development in the field of bioinorganic chemistry has also been the other important factor in spurring the growth interest in the complexes of the macrocyclic ligands.[7] Macrocyclic ligand systems often exhibit unusual properties and some times mimic related natural macrocyclic ligands are at the fore front of bioinorganic chemistry due to their variety of geometrical forms available and the possible encapsulation of the metal ion.[7]Organotin complexes have a range of pharmacological applications. The use of organotin(IV) halides as antiinflammatory agents against different types of oedema in mice has been reported.[8] Organotin(IV) complexes are also used in agriculture. They are efficient fungicides and bactericides.[9,10]

Rapidly expanding population and limited sources are thought to be the most pressing global problems today. This rapid increase in the world population has multiplied the benefits of economical and technological advancement. Fertility control is very essential for maintaining satisfactory standards in the developing countries. There is an increasing international recognition for the need to control human fecundity. Needless to say there is an immediate need for an inexpensive, safe and effective as well as universally acceptable contraceptive. For the evolution of such an ideal method for control of human fertility it is necessary that the reproductive process both of male and female need be more intensively investigated.

The male, an integral part of the family unit, has largely been sidelined by family planners. Currently, efforts are being made to develop a male contraceptive agent, which would inhibit fertility without affecting sex accessory function and libido. In this endeavor, a variety of synthetic compounds have been evaluated in males of laboratory species of mammals[11,12]. The results obtained are also encouraging. Therefore, this approach may form the basis for clinical regulation of male fertility in future. Inorganic compounds have also been investigated and applied for antifertility activity only and have not been screened for toxicological effect[13-16].

This paper describes the synthesis of the organotin(II) complexes derived from dicarboxylic acid with a view to studying the effect on antimicrobial activity and to explore the possibility of their use as potential therapeutic agents. The fungi and bacteria were selected in view of their economical importance. The compounds which showed good antimicrobial activity have been chosen for antifertility test in male rats.

## EXPERIMENTAL

All the solvents used were of high purity and distilled before use. $SnCl_2$ (BDH), malonic acid, succinic acid, glutaric acid and adipic acid (Fluka) and 1,4-diaminobutane (E. Merck) were used as obtained.

### Synthetic Procedure:

### *Synthesis of the complex [Sn(TAML$^1$)Cl$_2$]*

The reaction was carried out in 1:2:2 molar ratios. A magnetically stirred solution of $SnCl_2$ in methanol was added to a methanolic solution of 1,4-diaminobutane. The reaction mixture was stirred for 30 minutes and then the methanolic solution of malonic acid was added. The resultant mixture was stirred over night to yield solid product which was removed by filtration, washed several times with same solvent and vacuum dried. The compound was recrystallized in benzene. The purity of the products was checked by TLC on silica gel-G. However, if the same reaction is carried out under microwave conditions of green chemistry, the products were formed within 5-8 minutes and which is also an advantage of the new technique from the environmental as well as from the economic point of view. Same procedure has been used for the synthesis of [Sn(TAML$^2$)Cl$_2$], [Sn(TAML$^3$)Cl$_2$] and [Sn(TAML$^4$)Cl$_2$]. The reagents used were succinic acid, glutaric acid and adipic acid, respectively, in place of malonic acid.

### *Synthesis of the organotin(II) complexes*

The reaction was carried out in 1:1 molar ratio. In a Shlenk tube, a saturated methanolic solution of [Sn(TAML$^1$)Cl$_2$] was taken, stirred and pyridine was added. The stirred suspension was then cooled to -5$^o$C and stirred for 35 minutes. The sodium hydroxide (0.006 mol) followed by $CH_3I$ (0.003 mol) were added. The solution was gradually warmed to 20$^o$C and stirred further for 40 minutes. The solution was refluxed to half the volume, stirred, filtered and dried *in vacuo*. The physical properties of the complexes are given in Table 1.

**TABLE-1:PHYSICAL PROPERTIES AND ANALYTICAL DATA OF THE TIN(II) COMPLEXES.**

| Compound | M.P. °C and Colour | Analysis, Found (Calcd.) % | | | | | Mol. Wt. Found (Calcd.) |
|---|---|---|---|---|---|---|---|
| | | C | H | N | Cl | Sn | |
| $[Sn(TAML^1)Cl_2]$ | 225 White | 33.12 (33.50 | 4.46 (4.82) | 10.94 (11.16) | 23.49 (23.61) | 23.49 (23.61) | 504 (501.96) |
| $[Sn(TAML^2)Cl_2]$ | 201 White | 36.10 (36.26) | 5.16 (5.32) | 10.24 (10.57) | 22.14 (22.39) | 22.14 (22.39) | 521 (530.02) |
| $[Sn(TAML^3)Cl_2]$ | 219 White | 38.42 (38.74) | 5.36 (5.78) | 9.83 (10.04) | 21.06 (21.27) | 21.06 (21.27) | 549 (558.07) |
| $[Sn(TAML^4)Cl_2]$ | 198 White | 40.68 (40.98) | 5.82 (6.09) | 9.21 (9.56) | 20.08 (20.25) | 20.08 (20.25) | 579 (586.12) |
| $[CH_3Sn(TAML^1)C_5H_5N]$ | 235 Off white | 45.63 (45.74) | 6.70 (6.90) | 12.38 (13.33) | - | 22.15 (22.60) | 497 (525.14) |
| $[CH_3Sn(TAML^2)C_5H_5N]$ | 210 Off white | 47.66 (47.76) | 7.08 (7.28) | 11.80 (12.66) | - | 20.98 (21.45) | 528 (553.18) |
| $[CH_3Sn(TAML^3)C_5H_5N]$ | 219 Off white | 49.39 (49.59) | 7.52 (7.63) | 12.20 (12.04) | - | 20.04 (20.42) | 550 (581.20) |
| $[CH_3Sn(TAML^4)C_5H_5N]$ | 231 Off white | 51.25 (51.00) | 7.83 (7.94) | 10.68 (11.49) | - | 18.96 (19.48) | 585 (609.22) |
| $[C_2H_5Sn(TAML^1)C_5H_5N]$ | 227 Yellow | 46.01 (46.00) | 6.88 (6.98) | 11.92 (12.77) | - | 21.65 (21.09) | 519 (548.14) |
| $[C_2H_5Sn(TAML^2)C_5H_5N]$ | 215 Yellow | 48.50 (48.70) | 7.24 (7.46) | 11.48 (12.34) | - | 20.41 (20.92) | 548 (567.20) |
| $[C_2H_5Sn(TAML^3)C_5H_5N]$ | 220 Yellow | 50.24 (50.44) | 7.55 (7.78) | 10.55 (11.76) | - | 19.46 (19.94) | 563 (595.22) |
| $[C_2H_5Sn(TAML^4)C_5H_5N]$ | 238 Yellow | 52.00 (52.03) | 8.02 (8.08) | 10.44 (11.23) | - | 18.56 (19.04) | 599 (623.25) |

Same procedure has been used for the synthesis of $[RSn(TAML^n)C_5H_5N]$, where n = 2-4, R = $CH_3$ and $C_2H_5$. The reagents used were $[Sn(TAML^2)Cl_2]$, $[Sn(TAML^3)Cl_2]$ and $[Sn(TAML^4)Cl_2]$ with $CH_3I$ / $C_2H_5Br$.

***Physical measurements and analytical methods:***

The molecular weights were determined by the Rast Camphor Method. Conductivity measurements in dry DMF were performed with a conductivity Bridge type 305. Nitrogen and chlorine were estimated by the Kjeldahl's and Volhard's method, respectively. Tin was estimated as tin oxide gravimetrically. Infrared spectra of the precursors and their organotin(II) complexes were recorded in the range 4000-200 $cm^{-1}$ with the help of a Nicolet-Magna FTIR-550 spectrophotometer as KBr pellets. Multinuclear magnetic resonance spectra were recorded on an FX-90Q JEOL spectrometer operating at 90 MHz. $^1H$ NMR spectra were recorded in deuterated dimethylsulphoxide ($DMSOd_6$) at 89.55 MHz using tetramethylsilane (TMS) as an internal standard. $^{13}C$ NMR spectra were recorded in dry DMSO using TMS as the internal standard at 22.49 MHz. $^{119}Sn$ NMR spectra were recorded at 33.35 MHz using DMSO-$d_6$ as the solvent. The chemical shifts were determined, relatively to the external reference tetramethyltin. Carbon and hydrogen analyses were performed at Regional Sophisticated Instrumentation Center, Central Drug Research Institute, Lucknow.

**Bio-chemical Procedure:** The starting materials and their tin(II) complexes were evaluated for *in vitro* growth inhibitory activity against the phytopathogenic fungi *Fusarium oxysporium, Aspergillus niger* and against the bacteria *Staphylococcus aureus* and

*Pseudomonas cepacicola*. Adequate temprature, requisite nutrient, and growth media free from other microorganisms were employed for the growth of cultues of both the fungi and the bacteria.[35] The incubation periods for the fungi and bacteria were 96 h at 37°C and 24 h at 28°C respectively. The conventional fungicide 2-(methoxylcarbenyl) benzimidazol (Bavistin(R)) and the bactericide streptomycin were used as standards for comparing the activities of the compounds.

***Antifungal activity (Radial Growth Method):*** For the evaluation of the antifungal activity of the starting materials and their tin(II) complexes, these were dissolved at 50, 100 and 200 ppm concentrations in DMF and were then incorporated in potato-dextrose agar (PDA) medium against different types of fungus. The growth inhibition percentage was calculated on the basis of the average diameter of the fungal colony according to[17]

$$\text{Inhibition (\%)} = 100 ( \delta_C - \delta_T) / \delta_C \quad \ldots\ldots\ldots (3)$$

where $\delta_C$ and $\delta_T$ are the average diameters of the fungal colonies in the control plate and the test plate respectively.

***Antibacterial activity (Paper-Disc Plate Method):*** For the evaluation of antibacterial activity, a nutrient media containing 0.5% peptone, 0.15% yeast, 0.15% beef extract, 0.35% sodium chloride and 0.13% $KH_2PO_4$ in distilled water (1000 $cm^3$) was autoclaved for 20 min at 15 psi before inoculation. The compounds were dissolved in DMF at 500 and 1000 ppm concentrations. The 5 mm diameter Whatman No. 1 paper discs were soaked in different solutions of the compounds, dried and then placed in Petri plates previously seeded with the test organism. The plates were incubated for 24 h at 28°C and the inhibition zone around each disc was measured.

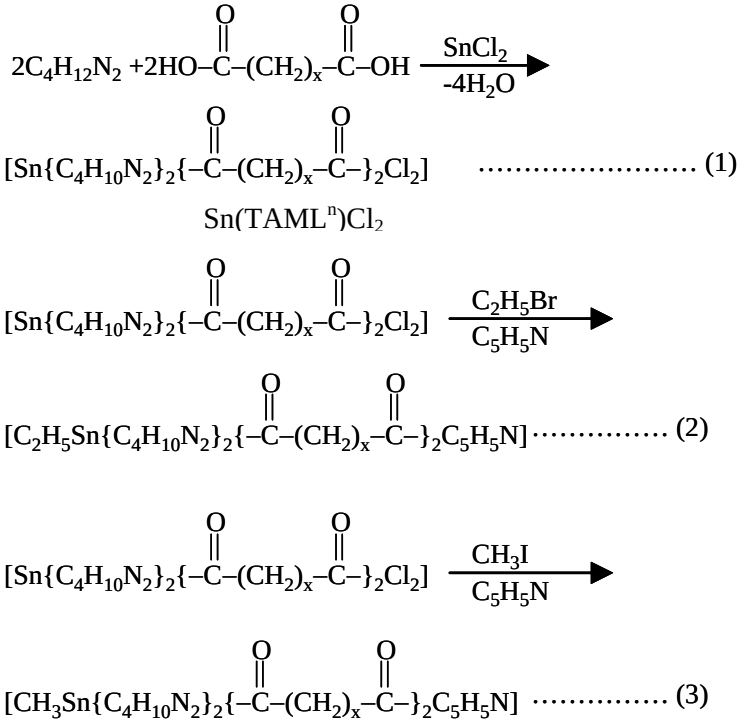
***Antifertility activity:*** The healty male albino rats (*Rattus norvegicus*) of Wister strain, weighing 200 to 220 gm were used in the present study. The animals were given free supply of commercial pelleted feed (Ashirwad Food Limited, Chandigarh, India) and tap water *ad libitum* and maintained under well-regulated light/dark schedule (14:10:L:D) at a temperature of 24 $\pm$ 3°C. The animals were divided into eight groups having 6 animals each. Group A animals were kept as control and were adminsitered olive oil where as animals of groups B, C, D and E were treated with tin(II) complexes (suspended in 0.2 ml olive oil) respectively at the dose level of 30 mg/kg between60 days.

The mating exposure test of all the animals was performed. They were cohabited with progesterone females in the ratio of 1:3. The vaginal plug and presence of sperm in the vaginal smear was checked for positive mating. Females were separated and resultant pregnancies were noted when dams gave birth. Fertility was calculated in control as well as treated groups. The animals were weighed and autopsied under light ether anesthesia. Sperm motility in cauda epididymis and density of testes and cauda epididymis suspended sperm were calculated.[18] The weight of testes, epididymis, ventral prostate and seminal vesicle were recorded and testes were frozen for measurements of glycogen, total protein, total cholesterol, sialic acid, acidic and alkaline phosphatase by the stanard laboratory techniques. Difference between control and treated groups were evaluated statistically using student's 't' test.[19] The data were expressed as mean $\pm$ SEM. Significance was set at $P \leq 0.01$ and $P \leq 0.001$.

## RESULTS AND DISCUSSION

The products formed are solids and soluble in methanol, benzene, carbontetrachloride, dimethylsulphoxide and dimethyl formamide. All these derivatives were purified by

crystallization. The purity was further checked by thin-layer chromatography using silica gel-G. It was observed that the spot moves as such for a particular type of compound which clearly indicates the purity of the compounds. The molecular weight determinations show these compounds to be monomers. The conductivity values measured for $10^{-3}$ M solutions in anhydrous DMF are in the range 13 – 32 $ohm^{-1}cm^2mol^{-1}$, showing them to be non-electrolytes. The condensation reactions of $SnCl_2$ with dicarboxylic acid and 1,4-diaminobutane was carried out in 1:2:2 molar ratios in dry methanol. The resulting new derivatives have been obtained as coloured solids. The methanolic solution of $[Sn(TAML^n)Cl_2]$ reacts with pyridine in the presence of sodium hydroxide and $C_2H_5Br$ / $CH_3I$. These reactions may be represented as follows:



Where n = 1, 2, 3 or 4 , x = 1, 2, 3 or 4

## Spectral Aspects

**IR Spectra:** The formation of the complexes has been revealed by the absence of $–NH_2$ stretching vibrations of the amino acid and OH groups of the dicarboxylic acids. The amide groups are present at 1640 – 1670, 1429 – 1485, 1238 – 1270 and 570 – 660 $cm^{-1}$ in the complexes.[20] It provides a strong evidence for the presence of a closed cyclic product. Strong and sharp absorption bands appearing in the regions 2830 – 2870 and 1410 – 1449 $cm^{-1}$ in the complexes were assigned to the C-H stretching and bending vibrational modes, respectively.[21] The presence of bands around 360 - 380 $cm^{-1}$ and 430 – 448 $cm^{-1}$ assignable to ν(Sn-Cl) and ν(Sn-N) vibrations respectively suggested that the amide nitrogen is coordinating to the tin ion.[22] A strong to medium intensity band appeared in the spectra of the complexes in the region 1195 - 1220 $cm^{-1}$ can be assigned to Sn-$CH_3$ stretching vibrations. The IR spectral data of the complexes are enlisted in Table 2.

**$^1$H NMR Spectra:** The $^1$H NMR spectra of the tin(II) complexes were recorded in DMSO-$d_6$ and the chemical shift values δ for the different protons are given in Table 3. The following

points, which confirm the suggested structures for the tin(II) complexes, are worth mentioning. The $^{1}H$ NMR spectra of the complexes do not show any signal corresponding to the amino and hydroxy groups. The broad signal observed in all the complexes at δ 7.90 – 8.17 ppm is due to the amide (CO-NH) protons.[23] In the spectra of the complexes, a multiplet observed in the region δ 1.97 – 2.09 ppm may be ascribed to the middle methylene protons of the 1,4-diamineobutane moiety (Table-3).

In the spectra of the complexes a multiplet arising due to the methylene protons (CO-N-$CH_2$) appeared in the region δ 3.32 – 4.49 ppm. Similar data have been reported by several authors[24,25] showing the presence of NH group in the macrocyclic ring system. Singlet appearing in the regions δ 2.86 – 2.90 and 3.05 – 3.10 ppm assigned to methylene protons of malonic and succinic acid moiety, respectively, while multiplets observed in the regions δ 3.20 – 3.23 ppm and δ 3.28 – 3.30 ppm are ascribed to the methylene proton of glutaric acid and adipic acid, respectively.The conclusions drawn from the IR and $^{1}H$ NMR spectra are in agreement with the $^{13}C$ NMR spectral data regarding the authenticity of the proposed structure. The shift observed in the carbons attached with nitrogen atoms is indicative of their coordination with the central tin atom (Table 4).

**TABLE-2:IR SPECTRAL DATA ($CM^{-1}$) OF THE TIN(II) COMPLEXES.**

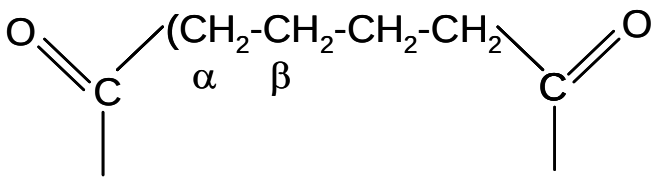
| Compound | Amide bands | | | | ν(NH) | ν(Sn-Cl) | ν(Sn-N) |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | | | |
| $[Sn(TAML^{1})Cl_2]$ | 1649 | 1425 | 1240 | 570 | 3275 | 365 | 441 |
| $[Sn(TAML^{2})Cl_2]$ | 1640 | 1470 | 1238 | 655 | 3278 | 380 | 435 |
| $[Sn(TAML^{3})Cl_2]$ | 1647 | 1429 | 1250 | 610 | 3219 | 360 | 442 |
| $[Sn(TAML^{4})Cl_2]$ | 1666 | 1480 | 1271 | 588 | 3256 | 375 | 430 |
| $[CH_3Sn(TAML^{1})C_5H_5N]$ | 1668 | 1439 | 1255 | 599 | 3247 | - | 434 |
| $[CH_3Sn(TAML^{2})C_5H_5N]$ | 1667 | 1442 | 1277 | 622 | 3238 | - | 440 |
| $[CH_3Sn(TAML^{3})C_5H_5N]$ | 1670 | 1459 | 1270 | 632 | 3274 | - | 443 |
| $[CH_3Sn(TAML^{4})C_5H_5N]$ | 1650 | 1485 | 1268 | 660 | 3266 | - | 432 |
| $[C_2H_5Sn(TAML^{1})C_5H_5N]$ | 1655 | 1468 | 1256 | 644 | 3210 | - | 448 |
| $[C_2H_5Sn(TAML^{2})C_5H_5N]$ | 1659 | 1475 | 1248 | 571 | 3172 | - | 435 |
| $[C_2H_5Sn(TAML^{3})C_5H_5N]$ | 1642 | 1478 | 1259 | 642 | 3190 | - | 445 |
| $[C_2H_5Sn(TAML^{4})C_5H_5N]$ | 1648 | 1481 | 1241 | 623 | 3199 | - | 440 |

**TABLE-3: $^{1}$H NMR (δ, PPM) SPECTRAL DATA OF THE TIN(II) COMPLEXES.**

| Compound | (CO-NH) | (CO-N-$CH_2$) | C-$CH_2$-C | CO-$(CH_2)_x$-CO |
|---|---|---|---|---|
| [Sn($TAML^1$)$Cl_2$] | 7.90 | 3.36 | 2.03 | 2.90 |
| [Sn($TAML^2$)$Cl_2$] | 8.06 | 3.32 | 2.05 | 3.05 |
| [Sn($TAML^3$)$Cl_2$] | 8.08 | 3.41 | 2.10 | 3.23 |
| [Sn($TAML^4$)$Cl_2$] | 7.99 | 3.44 | 1.97 | 3.28 |
| [$CH_3$Sn($TAML^1$)$C_5H_5N$] | 8.12 | 3.46 | 2.09 | 2.86 |
| [$CH_3$Sn($TAML^2$)$C_5H_5N$] | 7.98 | 3.40 | 2.04 | 3.07 |
| [$CH_3$Sn($TAML^3$)$C_5H_5N$] | 8.05 | 3.27 | 1.98 | 3.22 |
| [$CH_3$Sn($TAML^4$)$C_5H_5N$] | 8.17 | 4.49 | 2.08 | 3.28 |
| [$C_2H_5$Sn($TAML^1$)$C_5H_5N$] | 8.10 | 3.29 | 1.97 | 2.88 |
| [$C_2H_5$Sn($TAML^2$)$C_5H_5N$] | 7.99 | 3.32 | 2.09 | 3.10 |
| [$C_2H_5$Sn($TAML^3$)$C_5H_5N$] | 8.02 | 3.36 | 1.98 | 3.20 |
| [$C_2H_5$Sn($TAML^4$)$C_5H_5N$] | 7.98 | 3.38 | 1.99 | 3.30 |

**TABLE-4: $^{13}$C NMR SPECTRAL DATA (δ, PPM) OF TIN(II) COMPLEXES.**

| Compound | >C=O | >N-$CH_2$ | -$CH_2$-**C**-$CH_2$ | $C_\alpha$ | $C_\beta$ |
|---|---|---|---|---|---|
| 1 | 172.45 | 42.55 | 36.73 | 31.02 | 25.90 |
| 2 | 175.45 | 43.67 | 35.47 | 32.66 | 25.67 |
| 3 | 172.35 | 46.72 | 35.92 | 32.36 | 25.54 |
| 4 | 172.20 | 42.24 | 36.72 | 32.54 | 26.90 |
| 5 | 172.11 | 42.39 | 36.44 | 31.35 | 25.10 |
| 6 | 173.54 | 43.48 | 36.51 | 32.90 | 25.25 |
| 7 | 174.63 | 42.67 | 35.52 | 32.10 | 26.20 |
| 8 | 174.20 | 43.15 | 36.70 | 31.88 | 25.60 |
| 9 | 175.10 | 44.67 | 35.21 | 31.96 | 25.77 |
| 10 | 173.20 | 44.10 | 35.13 | 31.15 | 26.18 |
| 11 | 172.50 | 42.12 | 35.55 | 31.55 | 26.26 |
| 12 | 174.05 | 42.88 | 35.41 | 32.77 | 26.10 |




**$^{119}$Sn NMR Spectra**

$^{119}$Sn NMR spectra of the complexes give signals at δ 542 – 561 ppm, indicating coordination number six in these complexes around the tin atom.[26]

Thus, on the basis of the above spectal factors as well as on analytical data hexa-coordinated octahedral geometries have been established for the organotin(II) complexes.

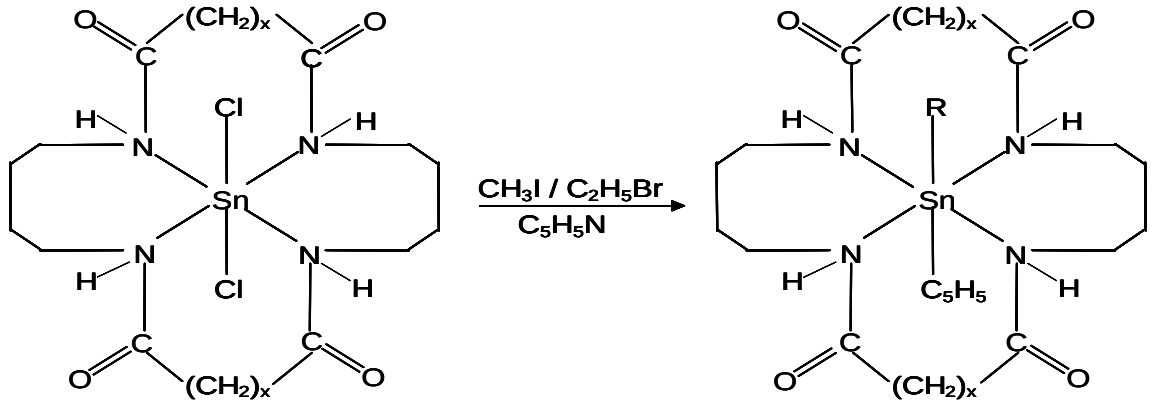



**Fig.1**

**Antimicrobial Screening**

*Mode of action*

Potato dextrose media (PDA) rich in carbohydrates as the major nutrient source is utilized by the microbes with the help of various enzymes (*viz.,* amylase, cellulase pectinase etc.). Metal based fungicides inhibit a wide range of enzyme involved in various metabolic pathways and ultimately causing cell death. Early work on the mode of action of fungicides showed that these compounds inhibit cell division. It was later[27,28] shown that the specific site of action is β-tubuline, a polymeric protein found in microtubules-essential component of the cytoskeleton. Phenyl and amine groups in the complexes effect nucleic acid, synthesis and mitochondrial electron transport also.

Chelation theory[29] accounts for the increased activity of the metal complexes. Chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and possible π electron delocalisation within the whole chelate ring. The chelation increases the lipophilic nature of the central atom which subsequently favours its permeation through the lipid layer of the cell membrane.

The results of fungicidal and bactericidal screening of the metal complexes against some pathogenic fungi and bacteria are recorded in Tables 5 and 6. The results show that the activity is enhanced on undergoing chelation. It is a well known fact that the concentration plays a vital role in increasing the degree of inhibition. As the concentration increases, the activity increased. The fungicidal activity was better as compared to the bactericidal activity.

**TABLE-5:ANTIFUNGAL SCREENING DATA OF THE STARTING MATERIAL AND THEIR TIN COMPLEXES. INHIBITION % OF THE 96 HOURS (CONC. IN PPM)**

| Compound | *Fusarium oxysporum* | | | *Aspergillus niger* | | |
|---|---|---|---|---|---|---|
| | 50 | 100 | 200 | 50 | 100 | 200 |
| $SnCl_2$ | 25 | 34 | 58 | 27 | 38 | 60 |
| Malonic acid | 21 | 33 | 58 | 28 | 40 | 66 |
| Succinic acid | 21 | 34 | 60 | 27 | 42 | 65 |
| Glutaric acid | 22 | 30 | 55 | 30 | 40 | 66 |
| Adipic acid | 24 | 35 | 61 | 32 | 48 | 67 |
| 1-4-Diaminobutane | 20 | 29 | 54 | 26 | 35 | 62 |
| $[Sn(TAML^1)Cl_2]$ | 74 | 80 | 86 | 83 | 88 | 92 |
| $[Sn(TAML^2)Cl_2]$ | - | - | - | 85 | 88 | 90 |
| $[Sn(TAML^3)Cl_2]$ | 79 | 84 | 86 | 81 | 87 | 91 |
| $[Sn(TAML^4)Cl_2]$ | 83 | 86 | 91 | 84 | 90 | 90 |
| $[CH_3Sn(TAML^1)C_5H_5N]$ | 80 | 88 | 91 | 88 | 92 | 95 |
| $[CH_3Sn(TAML^2)C_5H_5N]$ | 81 | 85 | 91 | - | - | - |
| $[CH_3Sn(TAML^3)C_5H_5N]$ | 86 | 97 | 98 | 92 | 95 | 96 |
| $[CH_3Sn(TAML^4)C_5H_5N]$ | 88 | 91 | 92 | 91 | 92 | 95 |
| $[C_2H_5Sn(TAML^1)C_5H_5N]$ | 90 | 92 | 95 | 93 | 92 | 95 |
| $[C_2H_5Sn(TAML^2)C_5H_5N]$ | 92 | 98 | 98 | 94 | 99 | 100 |
| $[C_2H_5Sn(TAML^3)C_5H_5N]$ | 90 | 92 | 95 | 93 | 92 | 95 |
| $[C_2H_5Sn(TAML^4)C_5H_5N]$ | 94 | 96 | 96 | 93 | 94 | 95 |
| Standard (Bavistin | 91 | 100 | 100 | 86 | 98 | 100 |

**TABLE-6:ANTIBACTERIAL SCREENING DATA OF STARTING MATERIALS AND THEIR TIN COMPLEXES (ZONE OF INHIBITION IN MM AFTER 24 HOURS 28 $\pm$ 2$^O$C, CONC. IN PPM).**

| Compound | Staphylococcus aureus (+) | | P. cepacicola (-) | |
|---|---|---|---|---|
| | 500 | 1000 | 500 | 1000 |
| $SnCl_2$ | 3 | 4 | 4 | 4 |
| Malonic acid | 7 | 6 | 3 | 4 |
| Succinic acid | 5 | 6 | 4 | 7 |
| Glutaric acid | 4 | 6 | 5 | 6 |
| Adipic acid | 5 | 6 | 4 | 6 |
| 1-4-Diaminobutane | 3 | 5 | 4 | 7 |
| $[Sn(TAML^1)Cl_2]$ | 8 | 11 | 8 | 13 |
| $[Sn(TAML^2)Cl_2]$ | 7 | 10 | 7 | 11 |
| $[Sn(TAML^3)Cl_2]$ | 9 | 12 | 10 | 14 |
| $[Sn(TAML^4)Cl_2]$ | 11 | 13 | 11 | 15 |
| $[CH_3Sn(TAML^1)C_5H_5N]$ | 9 | 12 | 13 | 17 |
| $[CH_3Sn(TAML^2)C_5H_5N]$ | 12 | 16 | 13 | 1 |
| $[CH_3Sn(TAML^3)C_5H_5N]$ | 13 | 17 | 16 | 18 |
| $[CH_3Sn(TAML^4)C_5H_5N]$ | 16 | 18 | 17 | 20 |
| $[C_2H_5Sn(TAML^1)C_5H_5N]$ | 11 | 13 | 12 | 14 |

| | | | | |
|---|---|---|---|---|
| $[C_2H_5Sn(TAML^2)C_5H_5N]$ | 13 | 15 | 13 | 16 |
| $[C_2H_5Sn(TAML^3)C_5H_5N]$ | 15 | 17 | 17 | 19 |
| $[C_2H_5Sn(TAML^4)C_5H_5N]$ | 16 | 18 | 17 | 20 |
| Standard (Bavistin | 15 | 17 | 2 | 5 |

From the bactericidal activity, it is apparent that the complexes were more toxic towards Gram(+) strains than Gram(-) strains. The reason is the difference in the structures of the cell walls. The walls of Gram(-) cells are more complex than those of Gram (+) cells. Lipopolysaccharides form an outer lipid membrane and contribute to the complex antigenic specificity of Gram(-) cells.

**Antifertility Activity:** Experiments were conducted *in vivo* in male rats to check antifertility activity and compounds that showed good antifungal and antibacterial activity were chosen for antifertility tests on male rats.

**Weight response:** There was no significant differences in the body weights. However, the weights of testes, epididymis, ventral prostate and seminal vesicle were decreased significantly ($P \leq 0.01$ to $P \leq 0.001$) after treatment with organotin complexes (Table 7).

**Sperm dynamics and fertility:** The sperm motility in cauda epididymis and sperm density in tests and cauda epididymis were decreased significantly after treatment with the complexes (Table 8).

**Biochemical findings:** The organotin complexes bring about a significant decrease in testicular total protein and total cholestrol contents. Where as a significant increase was observed in testicular glycogen, cholesterol, acid and alkaline phosphatase activity after treatment with the various compounds (Table 9).

The administration of the tin(II) complexes brings about a reduction in the weights of testes and sex accessories. The weight of testes is largely dependent on the mass of differentiated spermatogenic cells and the reduction in weight may be due to the decreased number of germ cell and elongated spermatids.[30] The observed reduction in the weight of accessory sex organs may be due to reduced bioavailability and the estrogenic and/or antiandrogenic activities of the compounds.[31] Low caudal epididymal sperm density may be due to alteration in androgen metabolism. The physiological and biochemical integrity of epididymis is dependent on androgens.[32] The 55 to 90% negative fertility test may be attributed to the lack of forward progression and reduction in the density of spermatozoa and altered biochemical milieu of cauda epididymis.[33] Treatment with the tin(II) complexes also changes the testicular biochemical parameters. Reduction in testicular sialic acid content may be due to the absence of spermatozoa of reduced androgen production.[34,35] Increased level of testicular cholesterol contents is attributed to the decreased androgen concentration which resulted in an impaired spermatogenesis.[36] An increase in the testicular glycogen of tin(II) complexes treated animals might be associated with poor utilization of glycogen due to a decrease in the phosphorylase activity.[37] Furthermore, an increase in the testicular acid and alkaline phosphatase activities indicate an impairment of the functional integrity of testes.[38,39]

## ACKNOWLEDGEMENT

One of the author (Ashu Chaudhary) is grateful to Prof. A.K. Singh, Department of Chemistry, Indian Institute of Technology Delhi, New Delhi for supporting this work and also thankful to Dr. Nisha Soni for interpreting the bio-chemical activity data.

## REFERENCES


1. R.R. Holmes, *Acc. Chem. Res.*, **22**, 190 (1989).
2. R.P.J. Williams, *Biochem. Soc. Trans.*, **18**, 989 (1990).
3. J.G.H. Dupreez, T.T.A. Gerber and O. Knoesen, *Inorg. Chem.*, **132**, 241 (1987).
4. M.W. Hosseini, J.M. Lehn, S.R. Duff, K. Gu and M.P. Mertes, *J. Org. Chem.*, **52**, 1662 (1987).
5. R.M. Izan, J.S. Brandshaw, S.A. Neilzen, J.D. Lamb, J.J. Christensen and D. Sen, *Chem. Rev.*, **85**, 271 (1985).
6. A.W. Herlinger, E.M. Funk, R.F. Chorak, J.W. Siebert and E. Roco, *Polyhedron*,**13**, 69 (1994).
7. G.A. Melson, *Coordination Chemistry of Macrocyclic Compounds*. Plenum Press, New York, **1** (1979).
8. G. Sembdner and B. Parthier, The Biochemistry and the Physicological and Molecular Actions of Jasmonates, *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **44**, 569 (1993).
9. F-Y. Tsai and C.H. Kao, Methyl Jasmonate Inhibits Endosperm Reserve Mobilization in Germinating Rice Seeds, *Physicol. Mol. Biol. Plants*, **2,** 67 (1996).
10. R. Ranjan and S. Lewak, Interaction of Jasmonic Acid and Abscisic Acid in the Control of Lipases and Proteases in Germinating Apple Embryos. *Physiol. Plant*, **93**, 421 (1995).
11. R. Kamal, R. Yadav and S.J. Sharma, *Phytother Res.*, **7**, 134 (1993).
12. R.S. Gupta, N. Sharma and V.P. Dixit, *An. Sci. Life*, **IX**, 224 (1990).
13. C. Saxena, R.V. Singh and S.C. Joshi, *Bull. Chem. Soc. Jpn.*, **67**, 1007 (1994).
14. K. Sharma, S.C. Joshi and R.V. Singh, *Metal Based Drugs*, **7**, 105 ( 2000).
15. M. Jain, S. Gaur, S.C. Diwedi, S.C. Joshi, A. Bansal and R.V. Singh, *Phosphorous Sulfur and Silicon*, **179**, 1517 (2003).
16. R.V. Singh, S.C. Joshi and R. Dwivedi, *Trans. Met. Chem.*, **29**, 70 (2004).
17. K.N. Thimmaiah, W.D. Lloyd and G.T. Chandrappa, *Inorg. Chim. Acta* **106**, 81 (1985).
18. M. Dudeja, R. Malhotra and K.S. Dhindsa, *Synth. React. Inorg. Met.-Org. Chem.*, **23**, 921 (1993).
19. J. Ipstein and Poly, in "Benchraft's Introduction to Biostatistics, **Vol. II**, Harper International, 1970, 44.
20. W.K. Pandey, O.P. Pandey, S.K. Sengupta and S.C. Tripathi, *Polyhedron*, **6**, 1611 (1987).
21. M.B.H. Hawlader, M.S. Islam and M.R. Karim, *Indian J. Chem.*, **39A**, 107 (2000).
22. A. Bansal and R.V. Singh, *Bol. Soc. Chil. Quim.* **45**, 479 (2000).
23. M. Shakir and S.P. Varkey, *Polyhedron*, **14,** 1117 (1995).
24. T.C. Woon and D.P. Fairlie, *Inorg. Chem.*, **31**, 4069 (1992).
25. I. Tabushi, Y. Taniguchi and H. Kato, *Tetrahedron Lett.*, **12**, 1049 (1977).
26. A, Bansal, N, Fahmi and R.V. Singh, *Main Group Met. Chem.*, **22**, 111 (1999).
27. J.A. Lucas, *Plant Pathology*, 3rd ed., **52** (1997).
28. E.C. Moore, M.S. Zeleck, K.C. Agarwal and A.C. Sartorelli, *Biochemistry*, **9**, 14492 (1970).
29. A. Kumari, R.V. Singh and J.P. Tandon, *Phosphorus, Sulfur and Silicon*, **66**, 195 (1992).
30. M.R.N. Prasad, N.J. Chinoy and K.M. Kadam, *Fertil. Steril.*, **23**, 186 (1972).
31. R.E. Chapin, M.W. Harris and S.M. David, *Fundam. Appl. Toxicol.*, **40**, 138 (1997).
32. N.C. Mills, *Int. J. Androl.*, **13**, 123 (1990).

33. De Brooks, In Spermatozoa (Eds.) De Fawcett and Badford, J.M. Urban and Schwargender, Baltimore, 23-24 (1979).
34. S.C. Joshi, R. Mathur, A. Gajraj and T. Sharma, *Environ. Toxicol. and Pharmacol.*, **14**, 91 (2003).
35. V.P. Dixit and R.S. Gupta, *Planta Medica*, **46**, 242 (1987).
36. N.J. Chinoy and Bhattacharya, *Indian J. Environ. Toxicol.*, **7(1)**, 12 (1997).
37. R.S. Bedwal, S.M. Edwards, M. Katoch, A. Bahuguna and R. Dewan, *Indian J. Exptl. Biol.*, **32(4)**, 243 (1994).
38. D.S. Gupta, P.K. Dutta, S. Saha and S. Samaddar, *Indian J. Exptl. Biol.* **19**, 689 (1981).
39. S. Sahdev, R. Bano and S.B. Lal, *Indian Environ. Toxicol.*, **6**, 1 (1996).

*(Received: 18 September 2007 Accepted: 18January 2008 RJC-106)*

**TABLE-7:EFFECTS OF TIN(II) COMPLEXES ON BODY AND REPRODUCTIVE ORGAN WEIGHTS ON MALE RATS.**

| Group | Treatment | Body weight (g) | | Organ weight (mg) | | | |
|---|---|---|---|---|---|---|---|
| | | Initial | Final | Testes | Epididymis | Seminal vesicle | Prostate |
| A | Control | 208.0 ± 15.2 | 230.0 ± 11.5[c] | 1350.0 ± 20.8 | 490.0 ± 20.3 | 520.0 ± 25.2 | 498.0 ± 10.2 |
| B | $[CH_3Sn(TAML^3)C_5H_5N$ | 210.0 ± 14.5 | 235.0 ± 15.5[c] | 1150.0 ± 25.3[a] | 380.0 ± 20.5[a] | 470.0 ± 20.5[a] | 435.0 ± 10.5 |
| C | $[C_2H_5Sn(TAML^3)C_5H_5N$ | 220.0 ± 11.8 | 238.0 ± 10.3[c] | 920.0 ± 15.1[a] | 350.0 ± 18.2[b] | 385.0 ± 17.2[a] | 390 ± 11.3[b] |
| D | $[CH_3Sn(TAML^4)C_5H_5N$ | 215.0 ± 11.8 | 239.0 ± 11.3[c] | 930.0 ± 30.7[b] | 320.0 ± 15.2[b] | 335.0 ± 15.5[b] | 310.0 ± 17.4[b] |
| E | $[C_2H_5Sn(TAML^4)C_5H_5N$ | 218.0 ± 15.3 | 240.0 ± 15.2[c] | 845.0 ± 17.3[b] | 310.0 ± 10.2[a] | 355.0 ± 15.4[a] | 340.0 ± 14.3[b] |

Groups B, C, D and E compared with Group A
Mean ± SEM of five animals a = ≤ 0.01
b = ≤ 0.001
c = ≤ ns

**TABLE-8:EFFECTS OF TIN(II) COMPLEXES ON BODY AND REPRODUCTIVE ORGAN WEIGHTS ON MALE RATS.**

| Group | Treatment | Sperm motility in cauda epididymis (%) | Sperm density million / $cm^3$ | | Fertility test (%) |
|---|---|---|---|---|---|
| | | | Testes | Cauda epididymis | |
| A | Control | 90.0 ± 5.0 | 5.1 ± 0.50 | 60.0 ± 3.5 | 100% positive |
| B | $[CH_3Sn(TAML^3)C_5H_5N$ | 50.0 ± 4.0[a] | 4.0 ± 0.30[a] | 48 ± 1.0[a] | 55% negative |
| C | $[C_2H_5Sn(TAML^3)C_5H_5N$ | 45.0 ± 3.0[b] | 3.8 ± 0.10[b] | 45 ± 1.5[a] | 60% negative |
| D | $[CH_3Sn(TAML^4)C_5H_5N$ | 35.0 ± 3.1[b] | 2.0 ± 0.13[b] | 31.0 ± 1.5[b] | 80% negative |
| E | $[C_2H_5Sn(TAML^4)C_5H_5N$ | 25.0 ± 2.0[b] | 1.8 ± 0.15[b] | 28.0 ± 2.0[b] | 90% negative |

Groups B, C, D and E compared with Group A
Mean ± SEM of five animals a = ≤ 0.01
b = ≤ 0.001

**TABLE-9:TESTICULAR BIOCHEMISTRY OF TIN(II) COMPLEXES.**

| Group | Treatment | Glycogen (mg/gm) | Total protein (mg/gm) | Total cholesterol (mg/gm) | Sialic acid (mg/gm) | Phosphatase mg/ip/g/h | |
|---|---|---|---|---|---|---|---|
| | | | | | | Acid | Alkaline |
| A | Control | 3.95 $\pm$ 0.57 | 220.0 $\pm$ 15.5 | 5.90 $\pm$ 0.26 | 5.40 $\pm$ 0.21 | 3.15 $\pm$ 0.19 | 10.50 $\pm$ 0.80 |
| B | $[CH_3Sn(TAML^3)C_5H_5N$ | 5.50 $\pm$ 0.10[a] | 150.0 $\pm$ 12.4[a] | 7.20 $\pm$ 0.20[a] | 3.50 $\pm$ 0.25[a] | 4.75 $\pm$ 0.15[a] | 12.50 $\pm$ 0.65[a] |
| C | $[C_2H_5Sn(TAML^3)C_5H_5N$ | 5.70 $\pm$ 0.15[a] | 140.5 $\pm$ 10.5[a] | 7.50 $\pm$ 0.15[a] | 3.20 $\pm$ 0.15[a] | 4.90 $\pm$ 0.12[b] | 17.20 $\pm$ 0.50[a] |
| D | $[CH_3Sn(TAML^4)C_5H_5N$ | 6.20 $\pm$ 0.18[b] | 120.5 $\pm$ 13.5[b] | 8.20 $\pm$ 0.13[b] | 3.29 $\pm$ 0.10[b] | 5.80 $\pm$ 0.11[b] | 16.80 $\pm$ 0.13[b] |
| E | $[C_2H_5Sn(TAML^4)C_5H_5N$ | 6.50 $\pm$ 0.16[b] | 125.8 $\pm$ 8.7[b] | 8.40 $\pm$ 0.18 | 2.90 $\pm$ 0.12$_b$ | 5.70 $\pm$ 0.12[b] | 17.88 $\pm$ 0.14[b] |

Groups B, C, D and E compared with Group A
Mean $\pm$ SEM of five animals a = $\leq$ 0.01
b = $\leq$ 0.001

**RJC** RASĀYAN JOURNAL OF CHEMISTRY
Vol.1,No.1(2008),1-14