Research Article

Synthesis, Spectroscopic Characterization, Antimicrobial, Pesticidal and *in Vitro* Antitumor Activity of Macrocyclic Complexes of Tin(II)

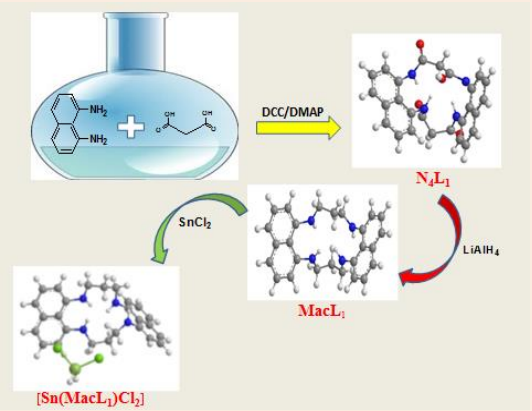
A. Chaudhary*[1], E. Rawat[1], S. Khan[2], P. Jindal[1], R. C. Kamboj[1] and R. V. Singh[3]

[1]Department of chemistry, Kurukshetra University, Haryana-136119, India
[2]Department of Chemistry, Banasthali University, Rajasthan-304001, India
[3]Department of Chemistry,University of Rajasthan, Rajasthan-302004, India

Abstract

An innovative approach for stepwise construction of functionalized macrocyclic complexes of tin(II) has been reported. Sixteen to nineteen membered tetraamidemacrocyclic ligands N_4L_1-N_4L_4 have been synthesized by the condensation of 1,8-diaminonapthalene with different dicarboxyclic acids in the presence of condensing reagent dicyclohexylcarbodiimide and 4-dimethylaminopyridine. On reduction, these macrocyclic ligands give a new series of tetraazamacrocycles $MacL_1$-$MacL_4$ which form complexes with tin(II) chloride. The ligands and their complexes were investigated using a combination of microanalytical, molecular weight determinations, IR, ^1H, ^{13}C and ^{119}Sn NMR spectral studies. The hexacoordinated state for tin has been confirmed by spectral studies. An octahedral geometry for these complexes has been proposed as the binding sites are the nitrogen atoms of the macrocycles. On the basis of chemical composition the representation of the complexes as $[Sn(MacL_n)Cl_2]$ (n=1-4) has been established.

Both the ligands and their complexes were found to possess appreciable antimicrobial and pesticidal activities. *In vitro* antitumor activities of these complexes have also been examined and the results were indeed very encouraging.



Keywords: Tetraazamacrocycles, Spectral, Antimicrobial, Pesticidal, Antitumor activities

***Correspondence**
A. Chaudhary,
Email: ashuchaudhary21@gmail.com

1. Introduction

Macrocyclic complexes of transition as well as non-transition metals occupy a unique segment of chemical space. Macrocycles have several features that make them interesting in efforts to tackle "difficult" targets with extended binding sites. Because of their size and complexity, they can engage targets through numerous and spatially distributed binding interactions, thereby increasing both binding affinity and selectivity. Furthermore, cyclization provides a degree of structural preorganization that may reduce the entropy cost of receptor binding compared to linear analogues [1].

In the past decade, their chemical diversity expanded significantly, supported by advances in bioinformatics and synthetic methodology. As a consequence, this structural type has now been successfully tested on most biological target classes. The goal of the present work is to put new perspectives into the current applications, opportunities, and challenges associated with synthetic macrocycles in drug discovery [2]. The award of the 1987 Nobel Prize in Chemistry to Pederson, Lehn and Cram is testimony to the importance of this rapidly expanding field [3]. Wherever possible, a comparison of macrocycles to relevant acyclic macrocycles has been seen [4]. An intriguing feature of

these systems is that the size of the ligands can be changed with relative ease by synthetic means. Template condensation lies at the heart of macrocyclic chemistry and is one of the most highlighted methods [5]. Metal macroyclic complexes enlarge the possibility of building up molecules better suited for binding to specific biological sites [6].

Tin(IV) complexes have attracted much interest because of their biological activities, in particular as potential biocides (e.g. antibacterial, antifungal) and anticancer agents [7]. Among main group metal compounds, they appear to exhibit the most potent antitumor activities, in some cases being more effective than *cis*-platin in vitro tests [8]. It is interesting to combine the anticancer properties exhibited by the tin(IV) complexes with the established biological effects. Tin(IV) complexes have received increasing attention owing to not only their potential applications in biotechnology, but also their fascinating chemical behaviour [9]. In general, the biochemical activity of organotin(IV) complexes is influenced greatly by the structure of the molecule and the coordination number of the tin atoms [10]. The eradication of fungi and bacteria still remains a challenging problem because of their emerging resistant powers. Encouraged by the above findings, the present article was directed to the synthesis of macrocyclic complexes of tin(II). Biological and inorganic aspects of the resulting complexes have been discussed.

2. Experimental

2.1. Synthesis

2.1.1. Chemicals

All the glass apparatus used during the experimental work were fitted with quick fit interchangeable standard ground joints. Melting points were determined in sealed capillary tubes. The solvents and volatile fractions were removed under reduced pressure using traps of conventional design dipped in ice. Fused calcium chloride towers were used to prevent the back diffusion of moisture from the vacuum pump. The chemicals including DCC, DMAP, $LiAlH_4$, $SnCl_2$, dicarboxylic acids and 1,8-diaminonapthalene were obtained from Sigma Aldrich and used as such.

2.1.2 Physical measurements and analytical methods

The molecular weights were determined by the Rast Camphor Method. Conductivity measurements in dry dimethylformamide 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 macrocyclic tin(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 a FX 90Q JEOL spectrometers operating at 90 MHz. ^{1}H NMR spectra were recorded in DMSO–d6 (deuterated dimethylsulphoxide) at 89.55 MHz using tetramethylsilane (TMS) as an internal standard. ^{13}C NMR were recorded in dry DMSO (dimethylsulphoxide) using TMS as the internal standard at 22.49 MHz. ^{119}Sn NMR spectra were recorded at 33.35 MHz using DMSO–d6 as the solvent. The chemical shifts were determined relative to the external reference tetramethyltin and are supposed to be accurate to ±1 ppm. Carbon and hydrogen analyses were performed at Regional Sophisticated Instrumentation Center, Central Drug Research Institute, Lucknow.

2.1.3. Synthesis of the Ligands N_4L_1-N_4L_4

The reaction is carried out in 2:2 molar ratios. The appropriate amount of dicyclohexylcarbodiimide (1.5369g) and catalytic amount of 4-dimethylaminopyridine in minimum amount of dichloromethane at 0°C, put on in magnetically stirred two necked round bottom flask. The reaction is followed by the addition of 1,8-diaminonapthalene (corresponding to the dicyclohexylcarbodiimide) in dichloromethane and malonic, succinic, glutaric or adipic acid (corresponding to the DCC) in dichloromethane. The resulting mixture was stirred for 10-12hrs at 0°C. The solid

product was isolated by filtration and washed several times with the same solvent and dry in vacuum. The solid products were recrystallized from benzene and dried in vacuum.

2.1.4. Synthesis of the Ligands $MacL_1$-$MacL_4$

The reaction is carried out in 1:2 molar ratios. The ligands N_4L_1-N_4L_4 (1g) were dissolved in tetrahydrofuran and cooled at 0°C. $LiAlH_4$ (corresponding to ligands) in tetrahydrofuran was stirred for about 10hrs in an ice bath. The reaction is followed by mixing the solution of ligand and $LiAlH_4$. The reaction mixture was stirred under reflux for 72hrs. After cooling it, 20mL of 15% aq. NaOH and then 30mL 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.

2.1.5. Synthesis of the Complexes $[Sn(MacL_1)Cl_2]$-$[Sn(MacL_4)Cl_2$

The reaction is carried out in 1:1 molar ratio 0.8-0.75g ligands $MacL_1$-$MacL_4$ were dissolved in methanol. The reaction is followed by the addition of Tin(II) chloride (corresponding to ligands $MacL_1$-$MacL_4$) solution. The resulting mixture was stirred for 12hrs at 0°C. The solid product was obtained by filtration and washed repeatedly with same solvent and dried in vacuo. The products were recrystallized from benzene.

2.2. Biological Assay

2.2.1. Microbiological Studies

2.2.1.1 Antifungal Activity

The antifungal activity of the standard fungicide (Bavistin), ligands and complexes were tested for their effect on the growth of microbial cultures and studied for their interaction with *Collectatrichum capsici* and *Macrophomina phaseolina* using Capek's agar medium having the composition, glucose 20 g, starch 20 g, agar-agar 20g and distil water 1000mL. To this medium was added requisite amount of the compounds after being dissolved in pet ether 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 amount of growth inhibition was calculated by the equation:

$$\text{Percent Inhibition} = (C\text{-}T) \times 100/C$$

Where, C and T are the diameters of fungal colony in control plate and test plates respectively [11].

2.2.1.2. Antibacterial Activity

Antibacterial activity was tested against *Pseudomonas capacicola (-) and Klebsella aerogenous (-)* by the Inhibition Zone Technique [12]. The nutrient agar medium having the composition peptone 5g, beef extract 5g, NaCl 5g, agar-agar 20g and distil water 1000mL was pipetted into the petridish. When it solidified, 5mL 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 bactericidal suspension. The compounds were dissolved in petroleum ether 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 petriplates 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 antibacterial activity of common standard antibiotic Streptomycin was also recorded using the same procedure as above at the same concentrations and solvent.

2.2.2. Pesticidal activity

For the past six decades we have been depended only on the synthetic pesticides for pest control. Chemical pesticides are one of the most important components of integrated pest management strategies in the profitable crop production. Pesticides are offering quick and accurate control of the crop pests. Pesticides can save farmers money by preventing crop losses to insects and other pests. To keep this view in the mind this experiment was conducted to determine pesticidal activities of the compounds.

The pesticidal activity of four ligands, N_4L_3, N_4L_4, $MacL_3$ and $MacL_4$ and two tin complexes, $[Sn(MacL_3)Cl_2]$ and $[Sn(MacL_4)Cl_2]$ has been conducted on *Chrotogonus trachypterus* pest. Bioactive experiment was conducted on their nymph, adult female and adult male (Figure 1). On basis of the mortality rate, the activity of the ligands and their tin(II) complexes was determined. *Chrotogonus tractypterus* (Blanchard) is most common polyphagous pest, which is found throughout the year [13]. Both the nymphs and adults cause cell damage to plants in its seedling stage.

A. Male **B**. Female **C**. Nymph

Figure 1 *Chrotogonus tractypterus*

Rearing of insects maintained in laboratory for bioassay. Insecticidal solutions in acetone were prepared from chemicals which were synthesized in laboratory for bio assay studies, against nymphs and adults *Chrotogonus tractypterus*. A thin layer was prepared in side of petridishes (10 cm in diameter) using 1 mol of solution in each part of the paired dish, after drying in room temperature for 1 hour -15 days old nymphs of *Chrotogonus tractypterus* were released in each petridish. Mortality counts were made after 24 hours. Thus, 10 replications of each concentration including control were run simultaneously. For adults topical method was used, 0.01mL of various solutions was introduced to intersegmental part of insect by microsyringe. The solutions of different concentration (250, 500 and 1000 ppm) of compounds were prepared by adding required quantity of compounds in acetone. These data were subjected to prohibit analysis [14] for calculation of LC_{50} values so as to final relative toxicity.

2.2.3. Antitumor activity

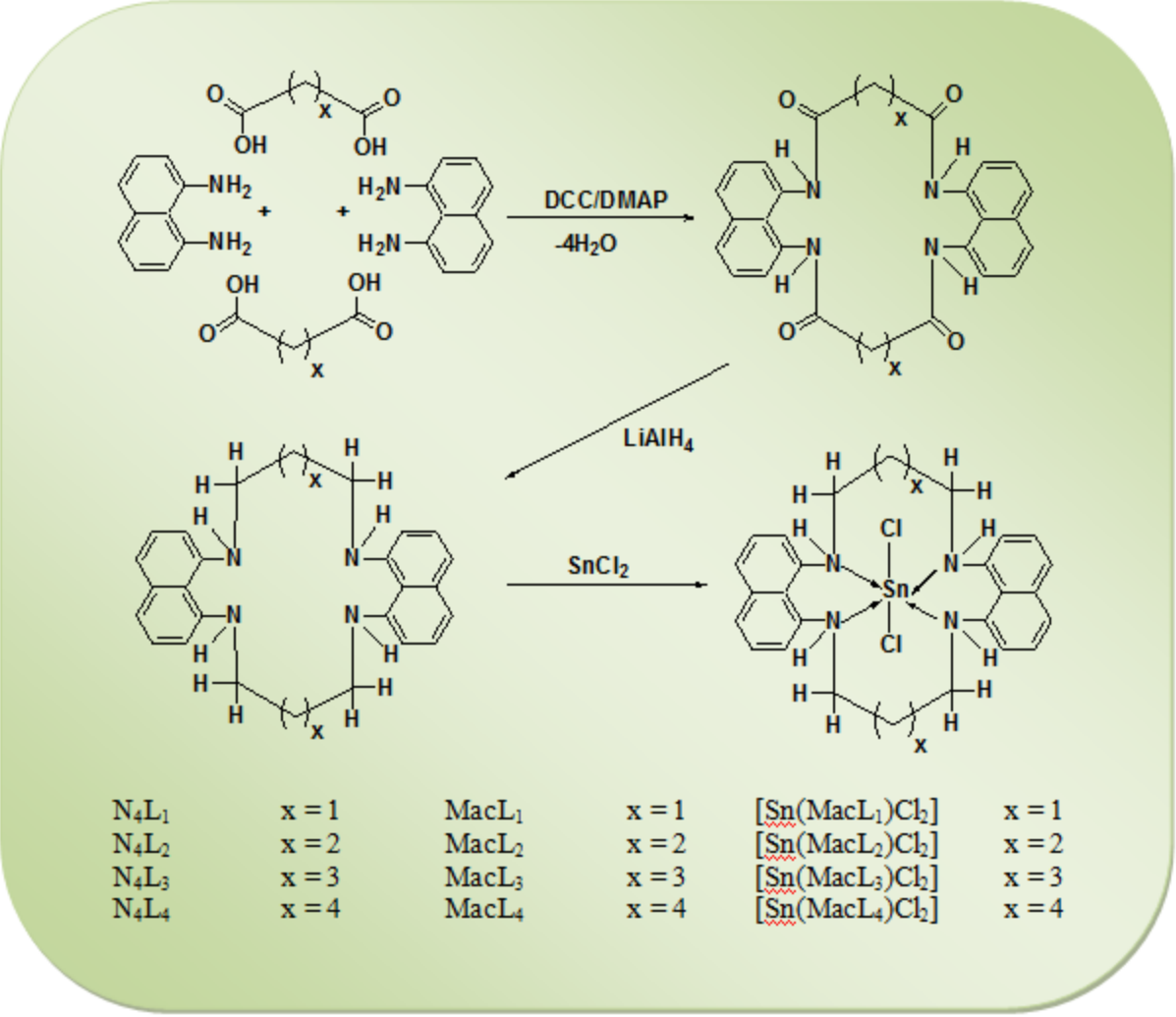
In vitro antitumor activity of synthesized compounds were evaluated against a panel of human cell lines, including human breast adenocarcinoma MCF-7, human colorectal cancer HT-29, human breast cancer MDA-MB-231 and human lung cancer NCI-H446 cell lines. The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately 4000 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The subject compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was

added to each well at a terminal concentration of 5mg/mL and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 mL DMSO each well and the absorbance at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software. The results were illustrated with Sorafenib as the positive control.

3. Results and discussion

3.1. Synthetic Route

A series of 16 to19 membered tetraazamacrocyclic ligands and their complexes $[Sn(MacL_n)Cl_2]$ (n=1-4) were derived by the condensation of different dicarboxylic acids with primary diamines in the presence of condensing reagents DCC and DMAP as shown in Scheme 1.



Scheme 1

The resulting macrocyclic complexes are colored solids, soluble in methanol and benzene but freely soluble in DMF, DMSO and THF. The conductivity values measured for 10^{-3} M solution in anhydrous DMF are in the range 13-22 ohm^{-1} cm^2 mol^{-1}, showing them to be non-electrolytes. Elemental analyses agree well with the stoichiometry and chemical formula of the compounds $[Sn(MacL_n)Cl_2]$. The physical properties and analytical data of the ligands and their tin(II) complexes are enlisted in Table 1.

Table 1 Physical properties and analytical data of tetraazamacrocyclic ligands and their tin(II) macrocyclic complexes

Compound	*Empirical Formula*	*M.P.(°C) and Colour*	*Analysis % Found (Calcd.)*			*Mol. Wt. Found (Calcd.)*
			N	*Cl*	*Sn*	
N_4L_1	$C_{26}H_{20}O_4N_4$	177 White	11.56 (12.38)	-	-	427 (452.46)
N_4L_2	$C_{28}H_{24}O_4N_4$	195 White	10.89 (11.65)	-	-	456 (480.52)
N_4L_3	$C_{30}H_{28}O_4N_4$	169 White	10.25 (11.01)	-	-	486 (508.57)
N_4L_4	$C_{32}H_{32}O_4N_4$	171 White	9.70 (10.44)	-	-	508 (536.62)
$MacL_1$	$C_{26}H_{28}N_4$	166 Light brown	13.81 (13.84)	-	-	383 (404.59)
$MacL_2$	$C_{28}H_{32}N_4$	154 Light brown	12.19 (12.94)	-	-	409 (432.65)
$MacL_3$	$C_{30}H_{36}N_4$	161 Light brown	11.32 (12.16)	-	-	438 (460.70)
$MacL_4$	$C_{32}H_{40}N_4$	178 Light brown	18.70 (11.46)	-	-	454 (488.75)
$[Sn(MacL_1)Cl_2]$	$C_{26}H_{28}N_4Cl_2Sn$	196 Off white	8.71 (9.42)	11.45 (11.93)	31.54 (31.91)	569 (594.21)
$[Sn(MacL_2)Cl_2]$	$C_{28}H_{32}N_4Cl_2Sn$	210 White	8.24 (9.00)	10.89 (11.39)	30.01 (30.47)	602 (622.27)
$[Sn(MacL_3)Cl_2]$	$C_{30}H_{36}N_4Cl_2Sn$	179 Off white	7.83 (8.61)	15.58 (10.90)	28.81 (29.15)	632 (650.32)
$[Sn(MacL_4)Cl_2]$	$C_{32}H_{40}N_4Cl_2Sn$	277 Off white	7.51 (8.25)	9.94 (10.45)	27.49 (27.95)	655 (678.37)

3.2. Spectral studies

3.2.1. Infrared spectra

The preliminary identification of the macrocyclic ligands and their complexes has been obtained from their infrared spectra. The first feature of all the complexes that attracts attention is the absence of –NH2 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 amide ligands N_4L_1-N_4L_4 in the region 3242-3270 cm^{-1} may be assigned to *v* (N-H) of amide group. The amide I, amide II, amide III and amide IV groups are present at 1655-1715, 1550-1592, 1252-1275, and 635-685 cm^{-1}, respectively [15,16]. It provides a strong evidence for the presence of a closed cyclic product. Strong and sharp absorption bands appeared in the regions 2820-3055 cm^{-1} and 1422-1450 cm^{-1} in all the complexes are assigned to the C-H stretching and C-H bending vibrational modes, respectively [17]. It has been noticed that tetraazamacrocycles $MacL_1$-$MacL_4$ do not show amide bands corresponding to tetraamidemacrocycles. However, a slight negative shift in the N-H stretching vibration has been observed. All other bands do not show appreciable change.

In the spectra of macrocyclic complexes $[Sn(MacL_1)Cl_2]$-$[Sn(MacL_4)Cl_2]$ as compared to their tetraazamacrocycles, the slight negative shift in the *v* (N-H) band which appeared in the region 3180-3220 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 441-485 cm^{-1} which is attributed to the Sn-N stretching vibrations [18]. The Sn-Cl stretching vibrations of compounds have been assigned at 485-496 cm^{-1} [19]. The infrared spectral data of the ligands and their complexes are listed in Table 2.

Table 2 IR spectral data (in cm^{-1}) of tetraamide & tetraazamacrocyclic ligands and their tin(II) macrocyclic complexes

Compound	*v (N-H)*	*C-H*		*v (Sn-N)*	*v (Sn-Cl)*
		Stretching	*Bending*		
N_4L_1	3242	3055	1450	-	-
N_4L_2	3245	3022	1447	-	-
N_4L_3	3270	3047	1443	-	-
N_4L_4	3265	3024	1448	-	-
$MacL_1$	3248	2892	1429	-	-
$MacL_2$	3243	2951	1438	-	-
$MacL_3$	3227	2903	1434	-	-
$MacL_4$	3233	2933	1441	-	-
$[Sn(MacL_1)Cl_2]$	3220	2852	1425	440	485
$[Sn(MacL_2)Cl_2]$	3210	2820	1422	459	488
$[Sn(MacL_3)Cl_2]$	3180	2864	1434	477	491
$[Sn(MacL_4)Cl_2]$	3184	2829	1427	466	490

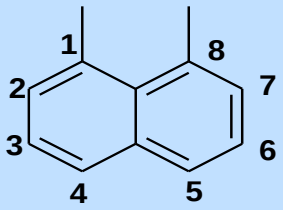
3.2.2. ^{1}H NMR Spectra

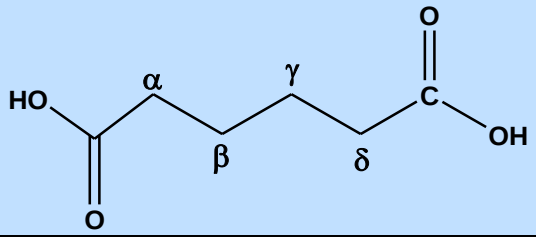
The ^{1}H NMR spectra of the amide ligands, tetraazamacrocycles and their macrocyclic complexes (Table 3) reveal the signals expected for the given Scheme 1. In the spectra of all the complexes, no band could be assigned for hydroxyl or amino groups, suggesting that the proposed macrocyclic complexes have formed after the condensation. A broad signal is observed in the region δ7.9-8.4 ppm for amide protons in macrocyclic ligands N_4L_1-N_4L_4. A singlet appearing in the region δ3.16-3.18 ppm and δ2.58-2.62 ppm may be ascribed to methylene protons of malonic acid and succinic acid. Multiplet appearing in the region δ2.05-2.23 and δ1.67-2.22 ppm may be assigned to methylene protons of glutaric and adipic acid moiety which are adjacent to the nitrogen atom. The ^{1}H NMR spectra of $MacL_1$-$MacL_4$ do not show any signal assignable to amide protons. It confirms the reduction of carboxylic groups. The spectra of tetraazamacrocycles show multiplet in the region δ3.7-4.1 ppm due to (C-NH) secondary amino protons. Signal observed in the regions δ3.01-3.06 ppm, which is attributed to the methylene protons (N-CH_2-C) of acid moiety. Another multiplet observed in the regions δ1.51-1.75 ppm and δ1.26-1.29 ppm, which is attributed to the methylene protons (N-C-CH_2 and N-C-C-CH_2)) of acid moiety. A comparative study of the ^{1}H NMR spectra of tetraazamacrocycles and their complexes showed the shift of the signals towards down field due to coordination of tin with nitrogen atoms.

Table 3 ^{1}H NMR spectral data of tetraamide & tetraazamacrocyclic ligands and their tin(II) macrocyclic complexes

Compound	**1,8-$C_{10}H_6$**	**(CO-NH)**	**CO-CH_2-CO**	**CO-$(CH_2)_2$-CO**	**CO-$(CH_2)_3$-CO**	**CO-$(CH_2)_4$-CO**
N_4L_1	8.12($H_{2,7}$b), 7.30 ($H_{4,5}$b), 7.13 ($H_{3,6}$b)	8.1	3.17$^{\alpha}$	-	-	-
N_4L_2	8.11($H_{2,7}$b), 7.28 ($H_{4,5}$b), 7.13 ($H_{3,6}$b)	8.1	-	2.61$^{\alpha/\beta}$	-	-
N_4L_3	8.08($H_{2,7}$b), 7.27 ($H_{4,5}$b), 7.15 ($H_{3,6}$b)	8.2	-	-	2.23$^{\alpha/\gamma}$, 2.05$^{\beta}$	-
N_4L_4	8.11($H_{2,7}$b), 7.27 ($H_{4,5}$b), 7.13 ($H_{3,6}$b)	7.9	-	-	-	2.22$^{\alpha/\delta}$, 1.67$^{\beta/\gamma}$
Compound	***1,8-$C_{10}H_6$***	***C-NH***	***N-CH_2-C***	***N-C-CH_2***	***N-C-C-CH_2***	
$MacL_1$	8.30($H_{2,7}$b), 7.29 ($H_{4,5}$b), 7.14 ($H_{3,6}$b)	4.0	3.06	1.75	-	
$MacL_2$	8.30($H_{2,7}$b), 7.28 ($H_{4,5}$b), 7.14 ($H_{3,6}$b)	3.9	3.02	1.52	-	
$MacL_3$	8.31($H_{2,7}$b), 7.27 ($H_{4,5}$b), 7.13 ($H_{3,6}$b)	4.0	3.05	1.51	1.29	
$MacL_4$	8.31($H_{2,7}$b), 7.28 ($H_{4,5}$b), 7.13 ($H_{3,6}$b)	4.1	3.06	1.52	1.27	
[Sn($MacL_1$)Cl_2]	8.31($H_{2,7}$b), 7.28 ($H_{4,5}$b),	4.1	3.05	1.74	-	

	7.13 ($H_{3,6}$b)				
	8.30($H_{2,7}$b),				
[Sn($MacL_2$)Cl_2]	7.28 ($H_{4,5}$b),	4.0	3.04	1.52	-
	7.13 ($H_{3,6}$b)				
	8.30($H_{2,7}$b),				
[Sn($MacL_3$)Cl_2]	7.27 ($H_{4,5}$b),	4.0	3.06	1.50	1.29
	7.13 ($H_{3,6}$b)				
	8.31($H_{2,7}$b)				
[Sn($MacL_4$)Cl_2]	7.27 ($H_{4,5}$b)	3.8	3.06	1.51	1.28
	7.13 ($H_{3,6}$b)				





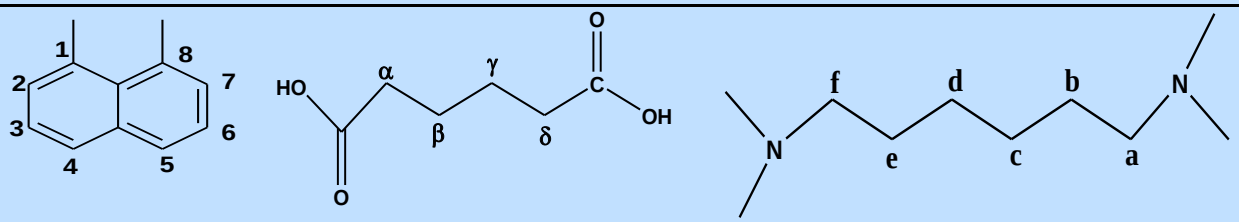
3.2.3. ^{13}C NMR spectra

The inferences 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 structures. The chemical shifts observed in the signals (Table 4) due to the different carbon atoms attached with the nitrogen atoms are indicative of their coordination with the central tin atom.

Table 4 ^{13}C NMR spectral data of tetraamide and tetraazamacrocyclic ligands and their tin(II) macrocyclic complexes

Compound	**1,8-$C_{10}H_6$**	**(CO-NH)**	**CO-CH_2-CO**	**CO-$(CH_2)_2$-CO**	**CO-$(CH_2)_3$-CO**	**CO-$(CH_2)_4$-CO**
N_4L_1	108.1 ($C_{2,7}$)	8.0	31.41$^{\alpha}$	-	-	-
	119.3 ($C_{4,5}$)					
	126.3 ($C_{3,6}$)					
	133.5 ($C_{1,8}$)					
N_4L_2	108.4 ($C_{2,7}$)	8.1	-	29.6$^{\alpha/\beta}$	-	-
	119.3 ($C_{4,5}$)					
	126.8 ($C_{3,6}$)					
	133.0($C_{1,8}$)					
N_4L_3	108.0 ($C_{2,7}$)	8.2	-	-	32.9$^{\alpha/\gamma}$,	-
	119.8 ($C_{4,5}$)				21.7$^{\beta}$	
	126.0 ($C_{3,6}$)					
	133.6 ($C_{1,8}$)					
N_4L_4	108.2 ($C_{2,7}$)	7.9	-	-	-	39.7$^{\alpha/\delta}$,
	119.6 ($C_{4,5}$)					25.5$^{\beta/\gamma}$
	126.3 ($C_{3,6}$)					
	133.0 ($C_{1,8}$)					

Compounds	1,8-$C_{10}H_6$	-N-$(CH_2)_6$-N-		
$MacL_1$	108.2 ($C_{2,7}$) 119.6 ($C_{4,5}$) 126.3 ($C_{3,6}$) 133.0 ($C_{1,8}$)	49.4($C_{a,c}$)	28.1(C_b)	-
$MacL_2$	108.2 ($C_{2,7}$) 119.6 ($C_{4,5}$) 126.3 ($C_{3,6}$) 133.0 ($C_{1,8}$)	51.7($C_{a,d}$)	33.5($C_{b,c}$)	-
$MacL_3$	108.2 ($C_{2,7}$) 119.6 ($C_{4,5}$) 126.3 ($C_{3,6}$) 133.0 ($C_{1,8}$)	44.7($C_{a,e}$)	29.4($C_{b,d}$)	23.3(C_c)
$MacL_4$	108.2 ($C_{2,7}$) 119.6 ($C_{4,5}$) 126.3 ($C_{3,6}$) 133.0 ($C_{1,8}$)	52.0($C_{a,f}$)	30.6($C_{b,e}$)	25.9($C_{c,d}$)
$[Sn(MacL_1)Cl_2]$	108.2 ($C_{2,7}$) 119.6 ($C_{4,5}$) 126.3 ($C_{3,6}$) 133.0 ($C_{1,8}$)	49.0($C_{a,c}$)	28.3(C_b)	-
$[Sn(MacL_2)Cl_2]$	108.2 ($C_{2,7}$) 119.6 ($C_{4,5}$) 126.3 ($C_{3,6}$) 133.0 ($C_{1,8}$)	50.9($C_{a,d}$)	33.1($C_{b,c}$)	-
$[Sn(MacL_3)Cl_2]$	108.2 ($C_{2,7}$) 119.6 ($C_{4,5}$) 126.3 ($C_{3,6}$) 133.0 ($C_{1,8}$)	44.2($C_{a,e}$)	28.8($C_{b,d}$)	23.3(C_c)
$[Sn(MacL_4)Cl_2]$	108.2 ($C_{2,7}$) 119.6 ($C_{4,5}$) 126.3 ($C_{3,6}$) 133.0 ($C_{1,8}$)	51.7($C_{a,f}$)	31.0($C_{b,e}$)	25.6($C_{c,d}$)



3.2.4. X-ray powder diffraction

The possible lattice dynamics of the finely powdered $[Sn(MacL_4)Cl_2]$ type of product has been reported on the basis of X-ray powder diffraction studies. The results show that the compound belongs to an 'orthorhombic' crystal system, having unit cell parameters of a = 8.73, b = 24.75 Å, C =23.8 Å, Z = 4, v = 2709.48 Å and ρ=1.707 cm^{-1} (Table 5). On the basis of above studies an octahedral environment around the metal atoms has been proposed.

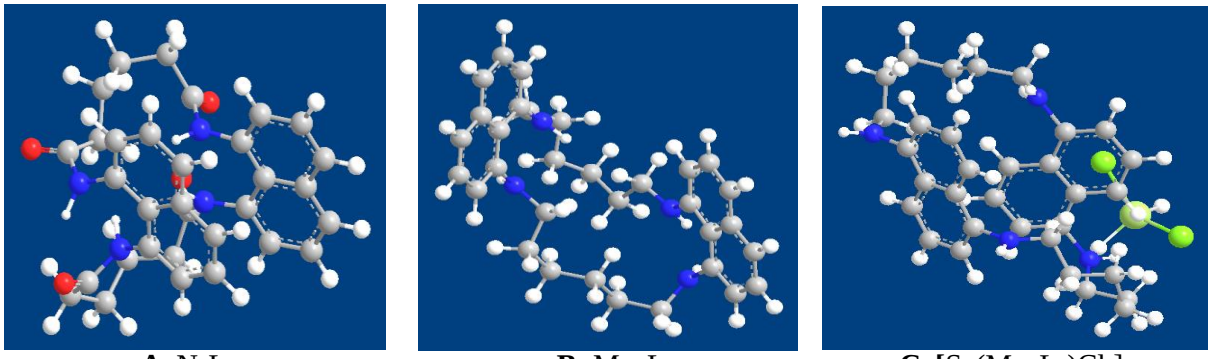
Table 5 X-ray powder diffraction data for [$Sn(MacL_4)Cl_2$]

Peak no.	d calcd.	d obsd.	I obsd.	H	k	l
1	11.95	11.95	95	0	1	1
2	8.05	7.98	47	0	3	0
3	7.72	7.70	27	1	1	0
4	7.02	7.09	44	1	0	1
5	6.03	6.03	36	0	4	0
6	5.73	5.84	28	0	3	0
7	4.8	4.84	28	0	5	0
8	4.85	4.89	100	1	4	0
9	4.16	4.19	17	1	5	0
10	3.86	3.88	22	2	1	1
11	3.49	3.50	63	1	6	1
12	3.22	3.22	16	2	3	2
13	3.18	3.19	26	1	1	4
14	3.02	3.02	24	1	4	0
15	2.71	2.71	21	3	0	0
16	2.65	2.66	17	3	1	1
17	2.53	2.53	20	3	3	1
18	2.12	2.12	10	3	1	4
19	2.17	2.17	19	1	2	6
20	2.20	2.21	16	2	3	2
21	2.14	2.14	19	2	8	3
22	2.19	2.16	17	2	3	5
23	2.24	2.23	14	2	2	5
24	2.33	2.35	14	3	1	3
25	2.40	2.39	16	1	2	2
26	2.40	2.41	18	3	3	2
27	2.51	2.52	17	1	9	1
28	2.58	2.58	20	2	5	3

a = 8.73A˚, b = 24.75A˚, c = 23.8A˚, Z = 4, V = 2709.48A˚ and ρ = 1.707 cm^{-1}

3.2.5. ^{119}Sn NMR spectra

The ^{119}Sn NMR spectrum of the complexes shows the signal at δ541.7-579.8 ppm which gives good agreement with a hexacoordinated tin [20]. Thus on the basis of the above discussion it seems that the macrocycles act as tetradentate chelating agents having four coordination sites, and hence a hexacoordinated environment around the tin atom has been proposed (Figure 2).



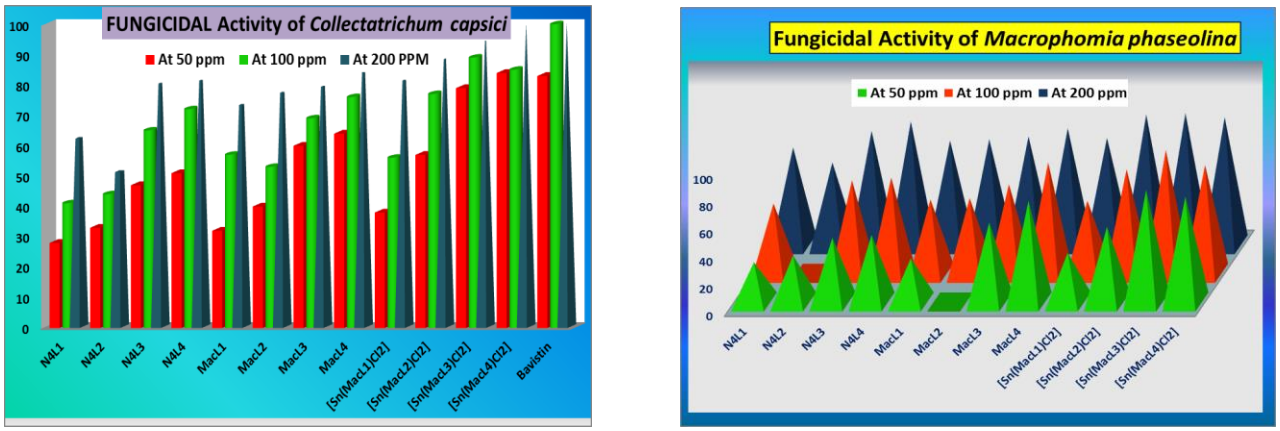
A. N_4L_4 **B.** $MacL_4$ **C. [**$Sn(MacL_4)Cl_2$]

Figure 2 MM2 structures of the compounds

3.3. Biological assay

3.3.1. Microbiological Studies

The ligands and their tin(II) complexes were evaluated for their antimicrobial activity against two bacteria, *Pseudomonas capacicola (-)* and *Klebsella aerogenous (-)* and two fungi, *Collectatrichum capsici* and *Macrophomia phaseolina*. The results were compared with those of the standard Bavistin for fungi and Streptomycin for bacteria. Both the ligands were found potentially active against the fungal and bacterial strains. The antimicrobial screening data show that both the ligands and their complexes individually exhibited varying degrees of inhibitory effects on the growth of the tested bacterial and fungal species. The antifungal (Figure 3) and antibacterial (Figure 4) results evidently show that the activity of the ligands became more pronounced when coordinated to the metal. The increased activity of the metal chelates can be explained on the basis of chelation theory [21], according to which the polarities [22,23] of the ligands and the central metal atoms are reduced through charge equilibration over the whole chelate ring. This increases the lipophilic character of the metal chelate and favors its permeation through the lipid layer of the bacterial membranes [24]. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or the difference in ribosomes of microbial cells. It has also been proposed that concentration plays a vital role in increasing the degree of inhibition; as the concentration increases, the activity increases.



A B

Figure 3 Fungicidal screening data of tetraamides, tetraazamacrocyclic ligands and their tin(II) complexes, percent growth inhibition after 4 days at 30±1°C

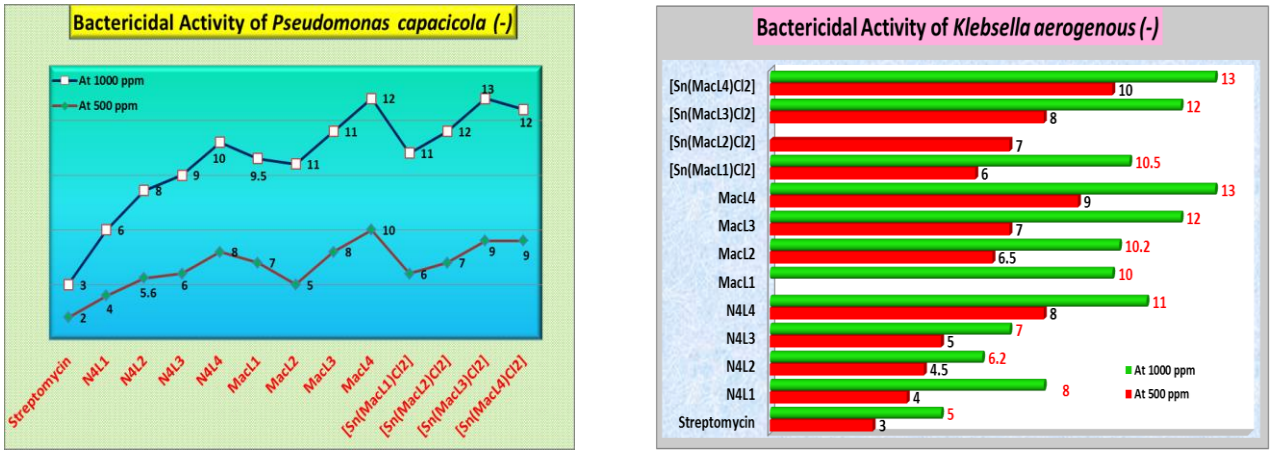
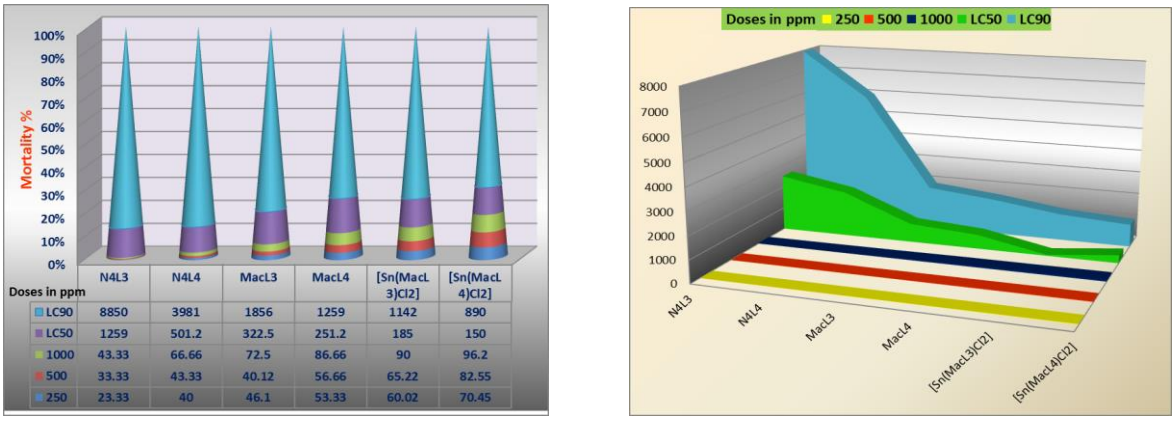


Figure 4 Bactericidal screening data of tetraamides, tetraazamacrocyclic ligands and their tin(II) macrocyclic complexes, diameter of inhibition zone (mm) after 24hrs at 28 ± 2°C (conc. in ppm)

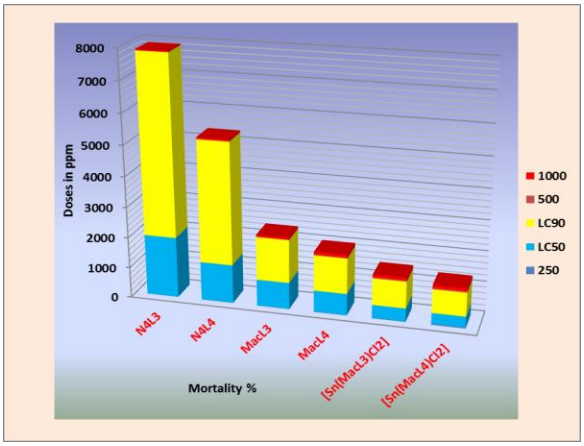
3.3.2. Pesticidal activity

The effect of compounds against 15 days old nymphs, adult female and adult male of *Chrotogonus tractypterus* were evaluated under laboratory conditions. The effect of the different concentrations on the larval mortality rate was examined at 72hrs after treatment. The results revealed that there was significant difference in percent mortality at different concentrations and it was noted that as concentration increases the larval mortality rate also increases. At 250 ppm concentration the complexes gave 60.02-70.45 percent mortality rate against 15 days old nymphs, 12.5-16.2 percent mortality rate against adult female, 22.5-46.2 percent mortality rate against adult male, at 500 ppm concentration the complexes gave 65.22-82.55 percent mortality rate against15 days old nymphs, 20.5-22.4 percent mortality rate against adult female, 40.5-44.4 percent mortality rate against adult male, and at 1000 ppm concentration the complexes gave 90-96.2 percent mortality rate against 15 days old nymphs, 71.5-84.2 percent mortality rate against adult female, 85.5-89.2 percent mortality rate against adult male, it was significantly superior over rest of the treatments. The treatment with acetone (control) gave no significant mortality of larvae of 15 days old nymphs, adult female and adult male of *Chrotogonus tractypterus*. The complexes were found to be more active against the pests used than the ligands themselves (Figure 5). The evaluation of pesticidal studies further revealed that pesticidal toxicity of the complexes also depends on the nature of the ligands. The pesticidal activity of the $MacL_4$ complexes is slightly higher than that of $MacL_3$ complexes.



A. 15 days old numph

B. Adult female (Balchand)



C. Adult male (Balchand)

Figure 5. Pesticidal effect of the ligands, tetraazamacrocyclic ligands and their complexes (doses in ppm)

3.3.3. Cytotoxic activity

Superficial growth inhibition against MCF -7, HT-29, NC1-H446 and MDA-MB-231 cell lines was observed for the ligands N_4L_1- N_4L_4, $MacL_1$-$MacL_4$ and the complexes $[Sn(MacL_1)Cl_2]$- $[Sn(MacL_4)Cl_2]$. Among them tin complexes exhibit more potent antitumor activities against MCF-7 and HT-29 and MB-231 cell lines than Sorafenib. Especially, $[Sn(MacL_3)Cl_2]$ and $[Sn(MacL_4)Cl_2]$ displayed excellent antitumor activities against MCF-7, HT-29 and MDA-MB-231 cell lines **(**Figure 6). Contrast to Sorafenib, the tested compounds displayed a broader spectrum of antitumor activity. However, Sorafenib exhibited weak activity on these three cell lines.

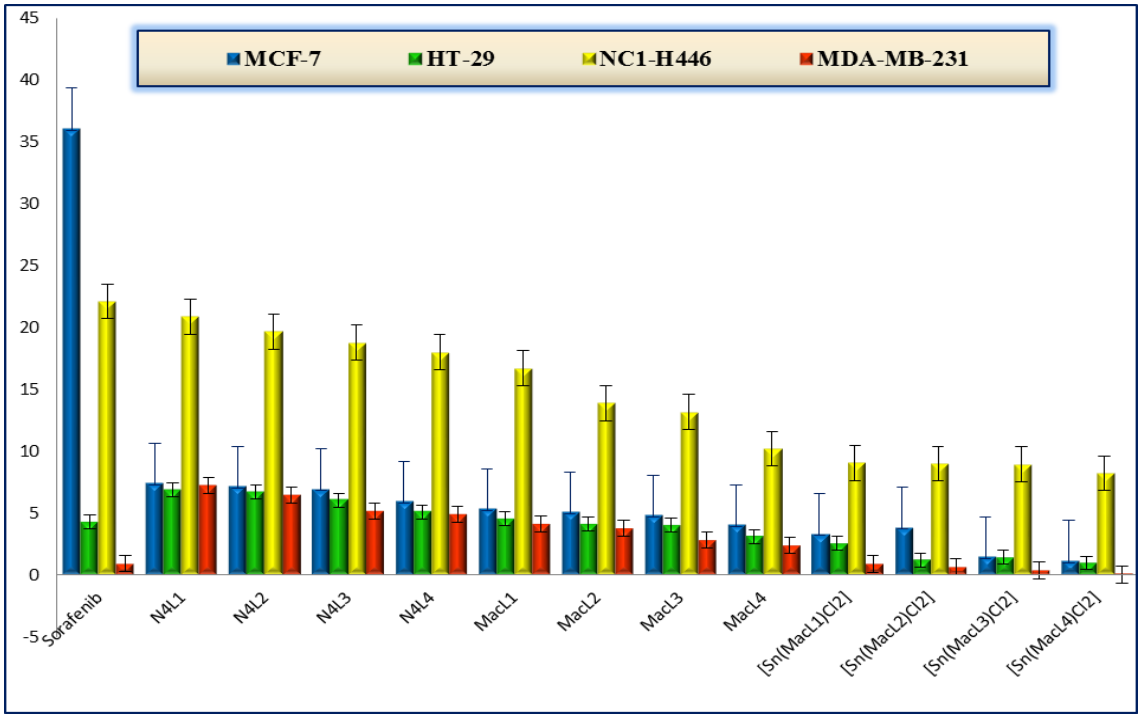


Figure 6 Growth inhibition against MCF -7, HT-29, NC1-H446 and MDA-MB-231 cell lines (IC_{50} (µMol/L)

Conclusions

Here, we propose a new approach for design of multitalented series of macrocyclic compounds. In our earlier communications [26-29] tetraoxotetraazamacrocyclic complexes of tin have been synthesized by the template condensation of metal salts, amines and dicarboxylic acid in 1:2:2 molar ratios. However, in the present paper tetraazamacrocycles have been isolated by employing different route, which established that dicyclohexylcarbodiamide (DCHC) and 4-dimethylaminopyridine (DMAP) can act as good condensing reagents. Initially tetraazamacrocyclic ligands were isolated by using amines and dicarboxylic acids in the presence of condensing reagents, DCHC and DMAP and reducing reagent $LiAlH_4$. On reaction with tin salts these tetraazamacrocyclic ligands give their corresponding complexes. An attempt to synthesize these ligands without the use of DCHC and DMAP failed to give these products and this is the fundamental difference between our present and previous studies. All the compounds showed appreciable antimicrobial activity. The newly designed compounds exhibited considerable pesticidal activity. The results analysis confirmed the cytotoxic potency of these compounds as compared to the standard, Sorafenib. Hence, there is sufficient scope for future studies.

DCC dicyclohexylcarbodiimide
DMAP 4-dimethylaminopyridine

Acknowledgements

The authors are grateful to **Professor Shyam Kumar**, Dean, Research and Development, Kurukshetra University, Kurukshetra, India, for his unwavering support. One of the authors (**Ashu Chaudhary**) wish to express her gratitude to the University Grants Commission (UGC), New Delhi, India for financial assistance in the form major research project vide letter no. F. No.42-231/2013 (SR) and F1-17-1/2012-13/MANF-2012-13-MUS-RAJ-9807.

References

[1] F. Giordanetto, J. Kihlberg, *J. Med. Chem.* **2014,** 57, 278.
[2] E. Marsault, M. L. Peterson, *J. Med. Chem.* **2011**, 54, 1961.
[3] Rajni, L. Singh, *International Journal of Advanced Engineering Applications* **2013**, 6(6), 74.
[4] J. Mallinson, I. Collins, *Future Med. Science* **2012**, 4(11), 1409.
[5] I. Masih, N. Fahmi, *Spectrochemica Acta Part A* **2011**, 79, 940.
[6] A. Alama, B. Tasso, F. Novelli, F. Sparatore, *Drug discovery today* **2009**, 14, 500.
[7] K. Sharma, S. Sharma, S. Gupta, *Int. J. Chem Tech Res.* **2013**, 5(1), 456.
[8] D. D. Qin, G. F. Qi, Z. Y. Yang, J. C. Wu, Y. C. Liu, *J. Fluoresc.* **2009**, 19, 409.
[9] H. Gaertner, R. Offord, P. Botti, G. Kuenzi, O. Hartley, *Bioconjugate Chem.* **2008**, 19, 480.
[10] M. N. Xanthopoulou, S.K. Hadjikakou, N. Hadjiladis, *Eur. J. Med. Chem.* **2008**, 43, 327.
[11] J. B. Chauhan, R. B. Subramanian, P. K. Sanyal., *Indian J. Environ. Toxicol.* **2002**, 12(1), 22.
[12] L. Dwara, R. V. Singh, *Appl. Organometal. Chem.* **2011**, 25, 643.
[13] P. Chakrabarti, *J. Mol. Biol.* **1993,** 234, 463.
[14] J. D. Cohen, R. S. Bandurski, *Planta* **1978**, 139, 203.
[15] M. Shakir, S. P. Varkey, *Polyhedron* **1995**, 14, 1117.
[16] M. Shakir, S. P. Varkey, O. S. M. Nasman, *Polyhedron* **1995**, 14, 1283.
[17] A. Chaudhary, N. Bansal, A. Gajraj, R. V. Singh, *J. Bioinorg. Chem.* **2003**, 96, 393.
[18] K. N. Thimmaiah, G. T. Chandra, W. D. Lloyd, *Inorg. Chim. Acta* **1985**, 107, 281.
[19] M. N. Patel, V. J. Patel, *Synth. React. Inorg. Met.-Org. Chem.* **2000**, 2, 407.
[20] A. Chaudhary, R. V. Singh, *Metal Based Drugs* **2002**, 8(6), 315.
[21] N. Raman, S. J. Raja, A. Sakthivel., *J. Coord. Chem.* **2009**, *62*, 691.
[22] R. S. Srivastava., *Inorg. Chim. Acta* **1981**, 56, 165.
[23] B. Keshavan, H. Kempe Gowda., *Turk. J. Chem.* **2002**, 26, 237.

[24] A. W. Varnes, R. B. Dodson, E. L. Wehry., *J. Am. Chem. Soc*. **1972**, 94, 946.
[25] A. A. F. Evans, *Ann. Appl. Biol.* **1973**, 75, 46.
[26] A. Chaudhary, A. Phor, R. V. Singh, *Indian J. Chem.* **2002**, 41A, 2536.
[27] A. Chaudhary, D. P. Jaroli, R. V. Singh, *Metal Based Drugs* **2002**, 8, 347.
[28] A. Chaudhary, N. Bansal, A. Gajraj, R. V. Singh, *J. Bioinorg. Chem.* **2003**, 96, 393.
[29] A. Chaudhary, A. Phor, S. Sharma, A. Gajraj, R.V. Singh, *Metal Based Drugs* **2002**, 9, 97.

Publication History
Received 12th June 2014
Revised 28th June 2014
Accepted 15th July 2014
Online 30th July 2014