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Research Article

MICROWAVE ASSISTED SYNTHESIS, ANTIMICROBIAL AND HEPATOTOXIC ACTIVITY OF TETRAAMIDE FUNCTIONALIZED MACROCYCLIC COMPLEXES OF TIN(II)

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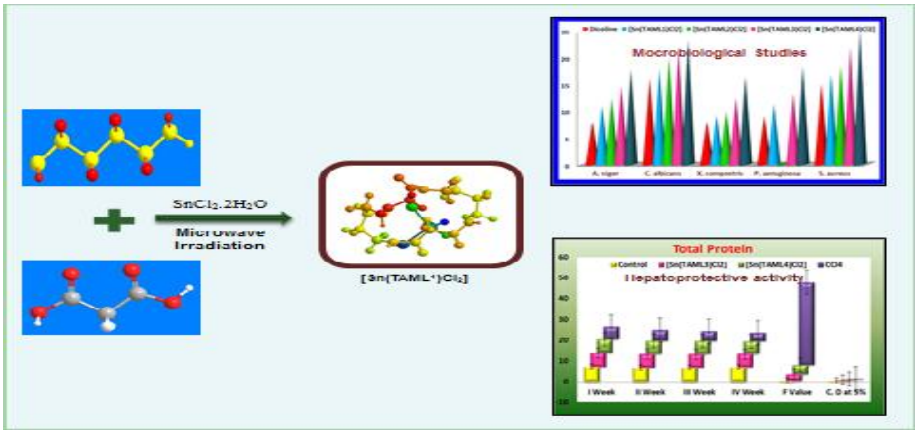
Abstract

Novel and efficient two-step microwave-assisted synthesis of biologically potent macrocyclic complexes of tin(II) have been reported. Reaction of $SnCl_2.2H_2O$ with macrocyclic ligand derived from 1,4-diaminobutane and different dicarboxylic acids yields the macrocyclic tin(II) complex with octahedral geometry. The complexes were investigated using a combination of microanalytical, molecular weight determinations, IR, ^{1}H, ^{13}C and ^{119}Sn NMR spectral studies. On the basis of chemical composition the representation of the complexes as $[Sn(TAML^{n})Cl_2]$ (n=1-4) has been proposed. The complexes were screened in vitro against a number of pathogenic fungi (*Aspergillusniger* NCIM 545 and *Candida albicans* NCIM 3471) and bacteria *(Xanthomonascompestris*, *Pseudomonas aeruginosa*NCIM2036, and *Staphylococcus aureus*NCIM2054)to assess their growth-inhibiting potential. The newly synthesized compounds displayed appreciable inhibitory potency in comparison with the standards. Hepatoprotectiveefficacy has also been carried out in male albino rats.



Keywords: Macrocyclic, microwave, antimicrobial, pathogenic, hepatoprotective.

Introduction

The advent of microwave assisted technology in organic chemistry dates back to the mid-1980s [1,2] and 1989 for inorganic synthesis [3]. Since the 1990s there has been a significant increase in the number of publications on microwave assisted reactions due to increased benefits associated with the reduced reaction time, improved yield and simplified procedures[4,5]. In addition, due to the environmental pollution and waste of resources, traditional chemical industry processes have also brought serious harm to the environment [6]. To

improve chemical processes and reduce environmental pollution has become an urgent task for chemical researchers. Microwave-assisted synthesis as a novel green chemical process shows distinct advantages over the traditional process, such as energy conservation, short reaction times, good conversions, and solvent-free mechanism [7]. Macrocyclic complexes attracted the attention of chemists and biologists because they are key building blocks for the synthesis of biologically active products bearing supramolecular skeletons. The discovery of a macrocyclic compounds was a milestone in chemistry [8]. Over the past several years, the prevalence of biologically active macrocyclesin medicinal chemistry literature has been increasing. Numerous recent research articles have discussed the role that macrocycles can play in medicinal chemistry; in particular looking beyond the established importance of natural product macrocycles in drug discovery [9,10].

Macrocyclic complexes have also received attention owing to their good antimicrobial activity. Concerning bacterial diseases, antibiotic research at the industrial level has been focused on the identification of more refined variants of already existing drugs [11]. Despite the rapidity with which new chemotherapeutic agents are introduced, bacteria have shown a remarkable ability to develop resistance to these agents and the search for new drugs, such as metal complexes is in progress [12]. There is also a pressing need for new antifungal agents because of the fast development of resistance of microorganisms to the state-of-the-art drugs currently used to treat different fungal growth. For this reason, the elaboration of new types of antifungal agents is presently a very real task [13].

In this paper we report the microwave synthesis, spectroscopic characterization and biological screening of macrocyclictin(II) complexes derived from 1,4-diaminobutane with different dicarboxylic acidsi.e. malonic, succinic, glutaric and adipic acids.

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Materials and Methods

Chemistry

Metal salt $SnCl_2.2H_2O$ as well as dicarboxylic acids i.e.malonic, succinic, glutaric and adipic acids were purchased from Sigma Aldrich and used as received. Solvents of analytical grade were distilled from appropriate drying agents immediately prior to use. 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. Molecular weights were determined by the Rast Camphor method. Nitrogen and chlorine were estimated by the Kjeldahl's and Volhard's method, respectively. Tin was estimated as tin oxide gravimetrically.Carbon and hydrogen analyses were performed at Regional Sophisticated Instrumentation Center, Central Drug Research Institute, Lucknow.

Infrared spectra of the precursors and their macrocyclictin(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 (deuterateddimethylsulphoxide) 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.

Microwave-assisted synthesis of $[Sn(TAML^{1})Cl_2]$-$[Sn(TAML^{4})Cl_2]$

The compound $[Sn(TAML^{1})Cl_2]$ was prepared by the condensation of 1,4-diaminobutane (2.4 mmol) and malonic acid(2.4 mmol), in presence of few drops of methanol (~3.0 mL) and irradiated by microwave irradiation for ~2 min. The resulting product (1.23 mmol) was again irradiated with $SnCl_2.2H_2O$ (1.23 mmol)for ~6 min. The resulting precipitate were recrystallized with alcohol and dried under vacuum. The compounds $[Sn(TAML^{2})Cl_2]$, $[Sn(TAML^{3})Cl_2]$ and $[Sn(TAML^{4})Cl_2]$ have also been obtained by the same procedure by using succinic, glutaric and adipic acids with 1,4-diaminobutane, respectively.

Conventional thermal method for synthesis of $[Sn(TAML^{1})Cl_2]$- $[Sn(TAML^{4})Cl_2]$

For comparison purpose, the above complexes have also been synthesized by the thermal method [14]. In this method, instead of few drops of methanol, 55-60 mL of methanol was used to dissolve the starting materials of the compound and the contents were refluxed for nearly 10-15 h. The residue formed was separated recrystallized from methanol and finally dried in vacuum over fused calcium chloride. A comparison between thermal method and microwave method is given in Table 1.

Biological evaluation

Microbiological studies

The dehydrated plate count medium (g/100 mL distilled water, glucose 0.1, yeast extract 0.25, tryptone 0.5) and

Sabouraud's dextrose agar (g/100 mL distilled water,glucose, peptone) were used for the antibacterial and antifungal activities, respectively. The target microorganisms included *Aspergillus niger* NCIM 545, *Candida albicans* NCIM 3471, *Xanthomonas compestris*, *Pseudomonas aeruginosa* NCIM2036, and *Staphylococcus aureus* NCIM2054. These strains were selected because they are routinely used in testing of disinfectants [15].

The stock cultures of these microorganisms were maintained at 20^{o}C in 15% glycerol [16]. The inoculum was prepared from stock cultures by a streaking onto the plate-count agar for bacteria and on Sabouraud's dextrose agar for fungi. After an overnight incubation, a single colony was used to inoculate sterile liquid media. The 5-mL broth was dispensed in the test tube and sterilized in the autoclave. The broths were then inoculated with respective cultures and incubated on an orbital shaker (150 ppm) overnight at 30^{o}C. A540 of bacterial cultures and *Candida albicans* were adjusted to 0.12 and 0.20, respectively. This corresponds to the 106–107 colony forming unit (Cfu/mL). The spore inoculum of *Aspergillus niger* containing 106 spores per mL was used. The solutions of the complexes were prepared in DMSO, added to the tube containing a 3mL liquid medium, and inoculated with 30 µL of the cultures. Incubation was done for 18 h at 37^{o}C.

Hepatotoxic Activity

The experimental rats (male albino rats) weighing 180-200g were divided into four groups of 10 each. The group I animals were treated as control. Hepatotoxicity was induced in the animals of group II, III and IV by oral administration of CCl_4 (0.25 mL/100 g body weight). Feeding was done biweekly for four weeks. From fifth day onwards, the group II and group III animals received an oral dose (50 mg/100 gbw for 30 days) of the compounds $[Sn(TAML^3)Cl_2]$ and $[Sn(TAML^4)Cl_2]$ respectively. All the animals were fed on commercial standard pellet diet (Hindustan Lever Ltd., Mumbai), water ad libitum and were maintained in the animal house (Zoology Department) at 25 ± 2^{o}C, 12 h light/dark cycle and 60±5% relative humidity.

Every week, three animals in each group were sacrificed and blood samples collected by direct heart puncture in to a sterilized dried centrifuge tube. Clear serum was collected and used for the assay of total bilirubin [17], total protein and albumin/globulin ratio [18]. The injury and disfunction of liver caused by the toxic effect of CCl_4 in experimental animals and similar results expected in the human viral hepatitis model [19]. In CCl_4-induced toxic hepatitis, a toxic reactive metabolite, trichloromethyl radical was produced by the microsomaloxidase system. This activated radical binds covalently to the macromolecules of the lipid membranes of endoplasmic reticulum and causes peroxidative degradation of lipids. As a result fats from the adipose tissue were translocated and accumulated in the liver [20]. In most of the studies this toxic chemical has been used as a tool to induce hepatotoxicity in experimental animals [21].

Results and Discussion

Chemistry

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 $[Sn(TAML^n)Cl_2]$. The physical properties and analytical data of the tin(II) complexes are enlisted in Table 2.

IR spectra

The most important IR absorption frequencies, along with the relative assignments of tin(II) complexes are summarized in Table 3. The significant changes with respect to the precursors are the absence of $-NH_2$ stretching vibrations of amino acids and -OH groups of the dicarboxylic acids. The spectra of all the complexes display absorption bands at 3265-3240 cm^{-1} assigned to ν (NH) of the amide group. The characteristic bands of the cyclic product appeared at 1700-1670, 1540-1460, 1270-1240 and 680-660 cm^{-1}arising from amide I, amide II, amide III and amide IV, respectively. Strong and sharp absorption bands appearing in the regions 2930-2890 $cm^{-1}$and 1450-1410 cm^{-1} in the complexes are assigned to the C-H stretching and bending vibrational modes, respectively [22]. Several new bands observed in the far IR region of the tin complexes [23,24] at 410-440 and 390-410 cm^{-1} are assigned to ν (Sn-N) and ν (Sn-Cl), respectively, suggesting that the amide nitrogen is coordinating to the tin metal.

^{1}H NMR spectra

The proton magnetic resonance spectral data of the tin complexes were recorded in DMSO-d_6. The chemical shift values relative to the tetramethylsilane (TMS) peak are listed in Table 4. The ^{1}H NMR spectra of the complexes do not show any signal corresponding to the amino and hydroxyl groups. The broad signal observed in all the complexes at δ 7.95 - 8.60 ppm is due to the amide (CO-NH) protons [25]. A multiplet observed in the region δ1.45-3.25 ppm may be ascribed to the middle

Table 1: Comparison between conventional and microwave methods of synthesis of the tin(II) macrocyclic complexes

Compounds	Compound Yield (%)		Solvent (mL)		Time	
	Thermal	Microwave	Thermal	Microwave	Thermal (Hrs.)	Microwave (Min.)
$[Sn(TAML^1)Cl_2]$	63	92	55	3	10	5.5
$[Sn(TAML^2)Cl_2]$	60	93	60	3	12	5.0
$[Sn(TAML^3)Cl_2]$	63	91	60	2	15	6.0
$[Sn(TAML^4)Cl_2]$	62	89	55	3	12	5.5

Table 2: The physical properties and analytical data of the tin(II) macrocyclic complexes

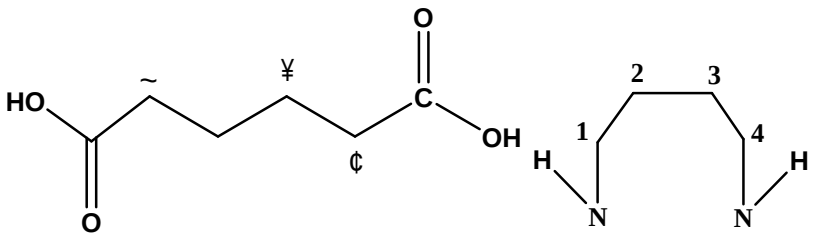
S. No.	Compound	M.P. (°C)	Colour	Analyses Found (Calcd.) %			Mol. Wt. Found (Calcd.)
				N	Cl	Sn	
1.	$[Sn(TAML^1)Cl_2]$	225	White	10.94 (11.16)	14.02 (14.13)	23.49 (23.61)	504 (510.96)
2.	$[Sn(TAML^2)Cl_2]$	201	White	10.24 (10.57)	13.16 (13.38)	22.14 (22.39)	521 (530.02)
3.	$[Sn(TAML^3)Cl_2]$	219	White	9.83 (10.04)	12.54 (12.71)	21.06 (21.27)	549 (558.07)
4.	$[Sn(TAML^4)Cl_2]$	198	Cream	9.21 (9.56)	11.78 (12.01)	20.08 (20.25)	579 (586.12)

Table 3: IR spectral data (in cm^{-1}) tin(II) macrocyclic complexes

Compound	¬ (NH)	Amide				¬ (Sn-N)	¬ (Sn-Cl)
		I	II	III	IV		
$[Sn(TAML^1)Cl_2]$	3265	1700	1460	1270	675	410	396
$[Sn(TAML^2)Cl_2]$	3252	1670	1490	1264	678	414	394
$[Sn(TAML^3)Cl_2]$	3244	1683	1510	1253	660	428	390
$[Sn(TAML^4)Cl_2]$	3240	1675	1540	1240	669	437	410

Table 4: ^1H NMR spectral data of tin(II) macrocyclic complexes

Compound	CO-NH	1,4 $-C_4H_8$	$COCH_2CO$	$CO(CH_2)_2CO$	$CO(CH_2)_3CO$	$CO(CH_2)_4CO$
$[Sn(TAML^1)Cl_2]$	8.0	3.20($H_{1,4}$), 1.55($H_{2,3}$)	2.09^{α}	-	-	-
$[Sn(TAML^2)Cl_2]$	8.1	3.20($H_{1,4}$), 1.52($H_{2,3}$)	-	$3.09^{\alpha,\beta}$	-	-
$[Sn(TAML^3)Cl_2]$	7.95	3.18($H_{1,4}$), 1.51($H_{2,3}$)	-	-	$2.18^{\alpha,\gamma}$, 1.85^{β}	
$[Sn(TAML^4)Cl_2]$	8.4	3.20($H_{1,4}$), 1.49($H_{2,3}$)	-	-	-	$2.16^{\alpha,\delta}$, $1.57^{\beta,\gamma}$



methylene protons [C-(CH_2)-C] of the 1,4-diaminobutane moiety. In the spectra of the complexesa multiplet arising due to the methylene protons (CO-N-CH_2) appears in the region δ1.40-3.49 ppm. Similar data have been reported by others [26,27] also. Singlets appearing in the regions δ2.0-2.11 and δ3.03-3.09 ppm have been assigned to the methylene protons of the malonic and succinic acid moieties, respectively. However, a multiplet observed in the regions, δ1.80-2.22 and δ1.56-2.27 ppm ascribed to the methylene proton of the glutaric and adipic acids, respectively.

^{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 (Table 5). The chemical shifts observed in the signals due to the different carbon atoms attached with the nitrogen atoms are indicative of their coordination with the central tin atom.

^{119}Sn NMR

^{119}Sn NMR spectra of the complexes give signals at δ598-623 ppm (Table 5), indicating the coordination number six in these complexes around the tin atom [28].
On the basis of the observed spectral evidences, an octahedral geometry (Fig. 1) has been proposed for the resulting complexes.

Biological assays

Microbiological studies

The newly designed compounds exhibited considerable extent of inhibition on both fungal and bacterial growth. The results analysis confirmed the potency of these compounds as compared to the standard (Fig. 2). Among these, compounds[$Sn(TAML^3)Cl_2$] and [$Sn(TAML^4)Cl_2$] showed highest activity, which was three folds more active than standard drug Dicoline.

Hepatoprotective activity

The biochemical parameters used are bilirubin, total protein, albumin, globulin, asparatate, aminotransminase, alanineamino transaminase and alkaline phosphatase for assessment of hepatoprotective activity in carbon tetrachloride induced toxicity. The major conclusions achieved by this experiment are as follows:

In the present study at the end of each week of the treatment, blood samples of the CCl_4 treated groups showed significant elevation in the levels of serum, total

bilirubin (1.80-3.70 mg/100 mL), serum globulin (2.50-2.80 mg/100 mL), aspartate aminotrasaminase(6.10-33.4 IU/100 mg of protein), alanine aminotransaminase (16.80-32.20 Int. units/100 mg of protein) and alkaline phosphatase (16.10-33.60 IU/100 mg of protein) activities as compared to the controls. These elevations are indicative of cellular leakage and loss of functional integrity of the cell membrane. On the contrary, serum total protein and albumin levels decreased from 5.98-4.19 and 3.50 – 1.35 mg/100 mL, respectively.

The estimation of serum total bilirubin confirms the intensity of jaundice. In normal population the serum bilirubin is in the range of 0.2 to 1 mg/100 ml of serum. In vital or toxic hepatitis the degree of excretion of bilirubin from the intestine is very less and bilirubin present in the liver is excreted into the canaliculi and then regurgitated into the blood stream. Hence hyperbilirubinemia is more common in hepatitis patients. At the end of each week of the treatment [$Sn(TAML^3)Cl_2$] and [$Sn(TAML^4)Cl_2$], significant reduced levels of serum total bilirubin (Fig. 3) in the blood samples even with the toxic effect of CCl_4 was observed. The results are similar to the reported by earlier workers [29].

Liver synthesizes a number of serum proteins is well known. The change in the serum protein levels forms the basis for important laboratory aids to diagnose the depth of jaundice. Further, there is a correlation between the degree of serum hypoalbuminemia and hyperglobulinemia. In normal population the albumin/globulin ratio is in the range of 2:1. The Fig. 4, depicts that in CCl_4-treated animals, serum total protein and albumin levels decreased with a moderate elevation in the levels of globuin. Even with the toxic effect of CCl_4, [$Sn(TAML^3)Cl_2$] and [$Sn(TAML^4)Cl_2$] were effective in restoring the levels of decreased serum total protein from 6.65-6.10 and 6.53-6.08 mg/100mL, respectively and levels of increased serum globulin from 2.45-2.55 and 3.53-2.75 mg/100 mL, respectively. The percentage of serum protein level restore was found to be more with [$Sn(TAML^3)Cl_2$].

The estimation of serum enzymes such as aspartate aminotrasaminase (ALT), alanine aminotransaminase (AST) and alkaline phosphatase (ALP) individually are helpful in the differential diagnosis of hepatic disease. The normal values of ALT, AST and ALP ranges from 5 to 20, 5 to 15 and 7 to 9 IU/mg of protein,

Table5: ^{13}C and ^{119}Sn NMR spectral data of tin(II) macrocyclic complexes

Compound	^{13}C NMR						^{119}Sn NMR
	CO-NH	1,4 –C_4H_8	$COCH_2CO$	$CO(CH_2)_2CO$	$CO(CH_2)_3CO$	$CO(CH_2)_4CO$	
$[Sn(TAML^1)Cl_2]$	169.4	39.20($H_{1,4}$), 28.55($H_{2,3}$)	37.8$^{\alpha}$	-	-	-	612
$[Sn(TAML^2)Cl_2]$	173.4	40.8($H_{1,4}$), 26.52($H_{2,3}$)	-	34.2$^{\alpha,\beta}$	-	-	598
$[Sn(TAML^3)Cl_2]$	174.7	41.18($H_{1,4}$), 25.51($H_{2,3}$)	-	-	37.9$^{\alpha,\gamma}$, 30.8$^{\beta}$		619
$[Sn(TAML^4)Cl_2]$	170.8	37.20($H_{1,4}$), 24.49($H_{2,3}$)	-	-	-	37.0$^{\alpha,\delta}$, 24.57$^{\beta,\gamma}$	623

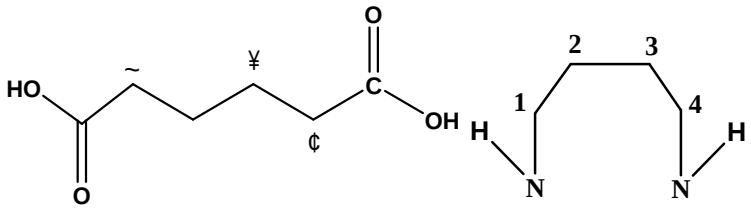


Figure 1: (A) Proposed structure of the tin(II) macrocyclic complexes (x=1,2,3 and 4) and (B) Energy minimized MM2 structure of $[Sn(TAML^1)Cl_2]$

A B

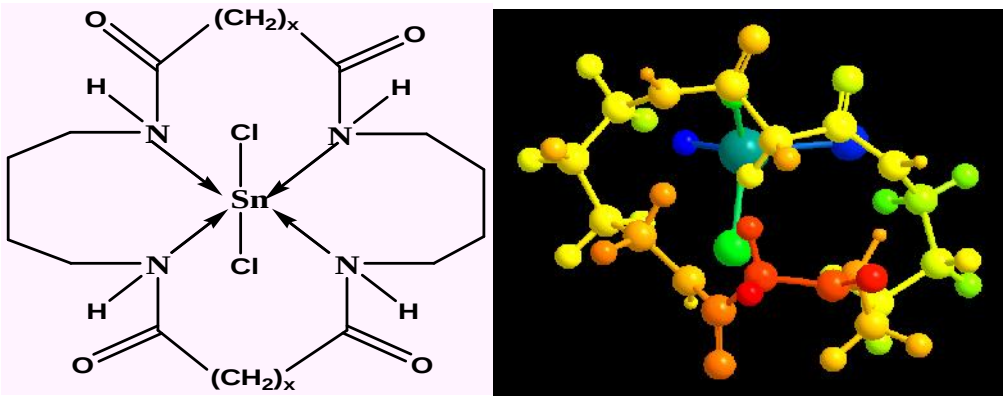


Figure 2: Antimicrobial Activity of the tin(II) macrocyclic Complexes

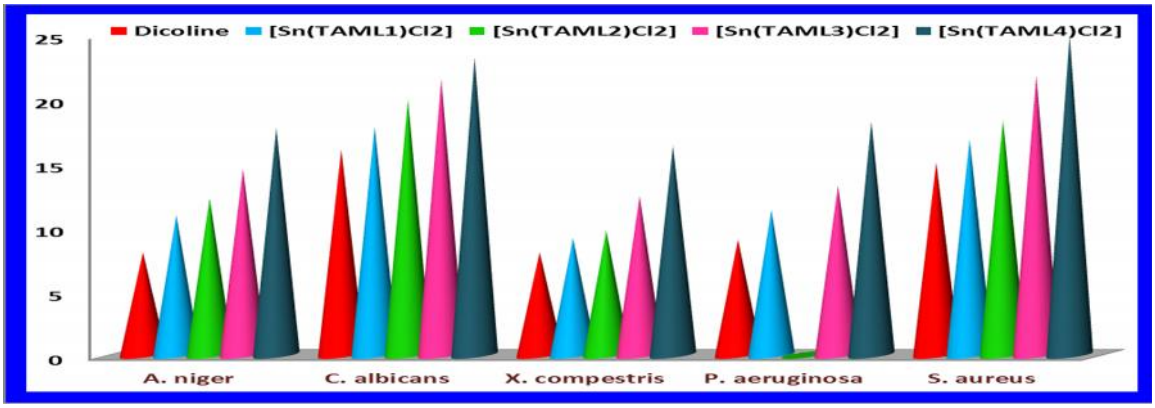


Figure 3: Total bilirubin levels in serum as compared to the controls

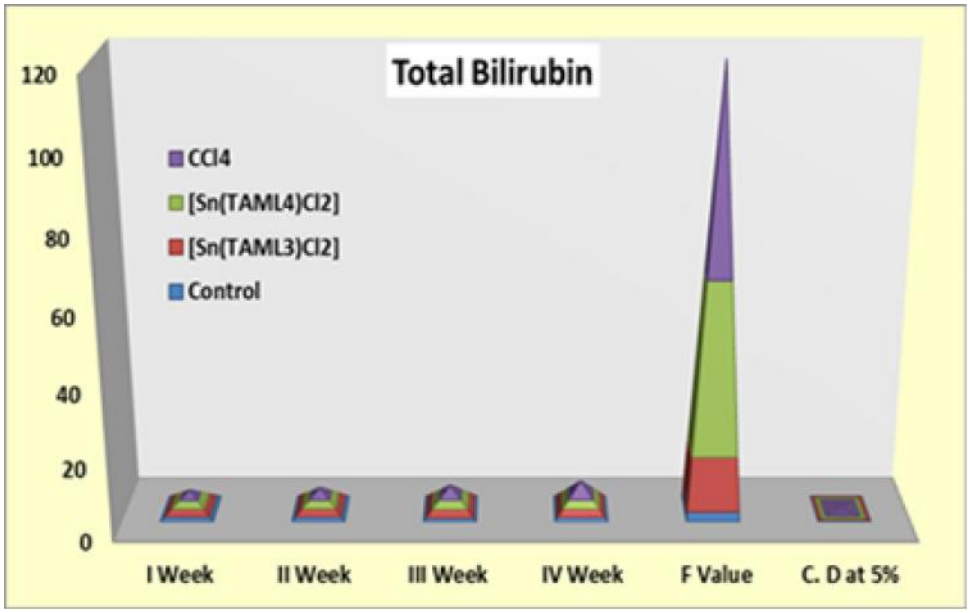
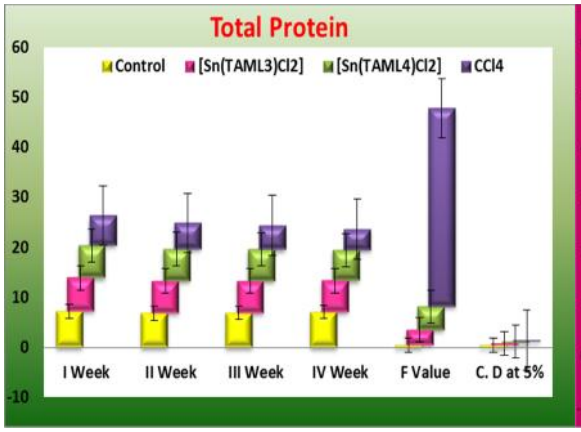


Figure 4: (A) Total protein (B) Albumin and (C) Globuin as compared to the controls

A



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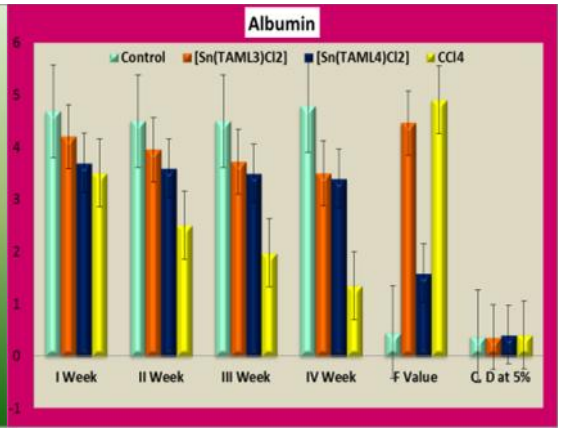


C

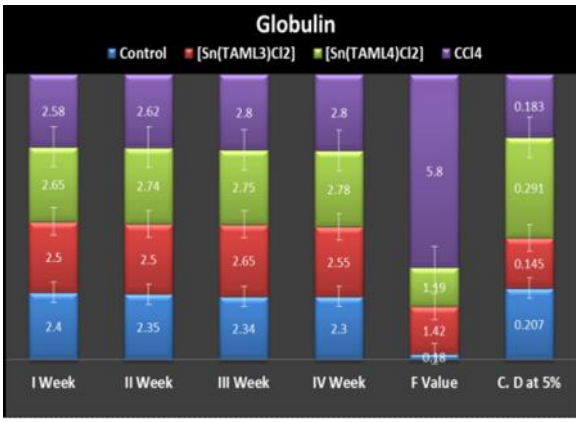
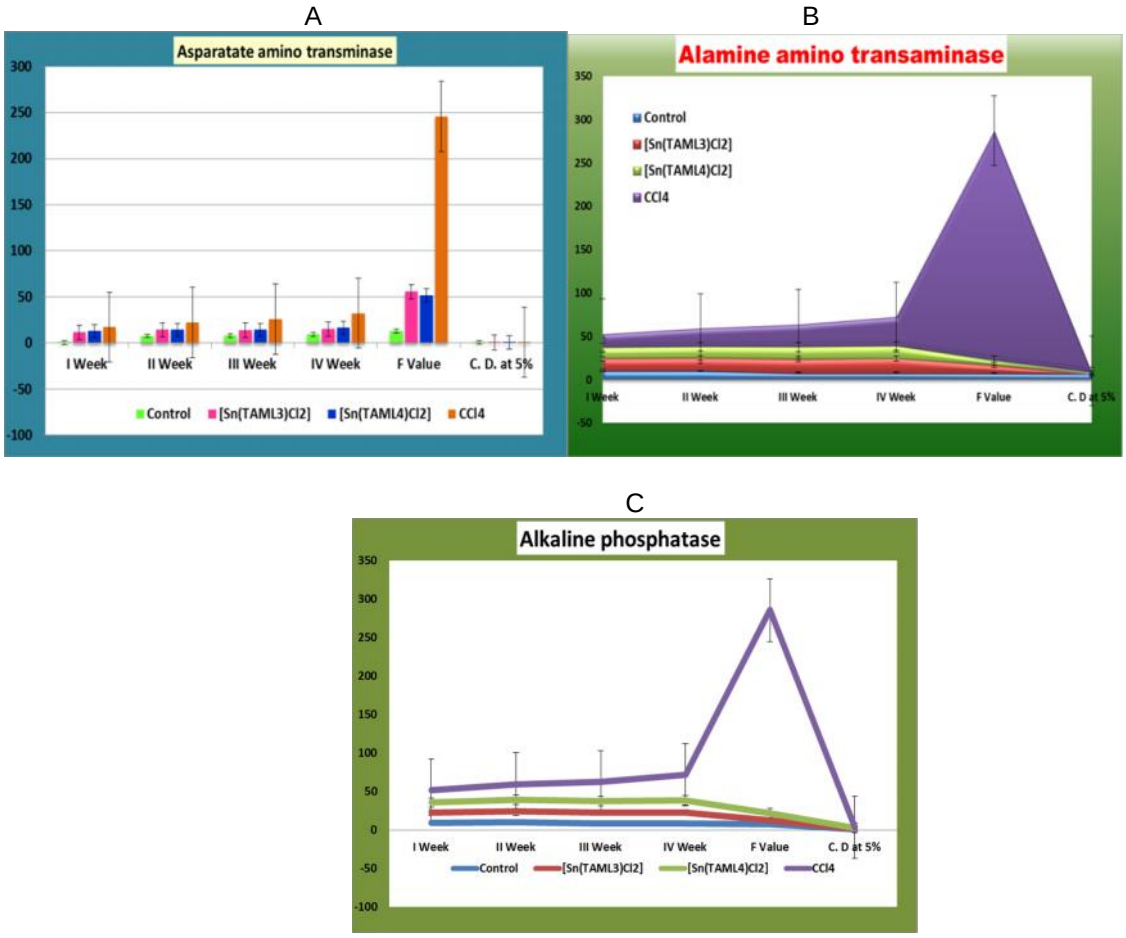


Figure 5: (A) Aspartate aminotrasaminase and (B) Alanine aminotransaminase (C) Alkaline phosphatase level as compared to the controls



respectively. The concentration of these enzymes increase in serum whenever the liver tissue was damaged(Fig. 5). It is presumably due to the release of enzymes from the damaged cells. In acute hepatic necrosis the level of AST and ALT are expected to increase by 2 to 20 folds over that of controls.

On the contrary, in obstructive and post hepatic jaundice elevation of ALP was more. In the present investigations, in CCl_4 treated group the activities of these enzymes increased parallelly from 1 to 4 weeks of treatments. Administration of $[Sn(TAML^3)Cl_2]$ and $[Sn(TAML^4)Cl_2]$ prevented the increase in the levels of these enzymes, showed the pattern of recovery from the toxic effect

Conclusion

A series of macrocyclic derivatives were designed and synthesized as multi-targeted agents for the treatment against microbial species i.e. fungi and bacteria and hepatotoprotective activity. The complexes inhibited the growth of test fungi (*Aspergillus niger* NCIM 545 and *Candida albicans* NCIM 3471) and bacteria *(Xanthomonas compestris*, *Pseudomonas aeruginosa* NCIM2036, and *Staphylococcus aureus* NCIM2054)to considerable degrees. Biochemical parameters also confirmed the promising hepatoprotective activity of the complexes. The breadth of these different biological applications for complexes is exciting, but also presents on going challenges.

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