Bioinorganic Relevance of Lead(II) and Tin(II) Complexes of 3,4,9,10-Tetraphenyl-1,2,5,6,8,11-Hexaazacyclododecane 7,12-Dithione-2,4,8,10-Tetraene

Ashu Chaudhary[a], Anita Phor[b] and R.V. Singh[*a]

[a]*Department of Chemistry, University of Rajasthan, Jaipur – 302 004, INDIA*
[b]*Department of Chemistry, Hindu College, Sonepat 131001, India*

ABSTRACT

A new class of lead(II) and tin(II) complexes of a macrocyclic Schiff base ligand containing a thiosemicarbazone moiety has been prepared with a general composition $[M(H_2MacL^2)X_2]$, where M = Pb(II) or Sn(II); H_2MacL^2 = 3,4,9,10-tetraphenyl-1,2,5,6,8,11-hexaazacyclododecane-7,12-dithione-2,4,8,10-tetraene; X = Cl^- or NO_3^-. When the mesocycle-6-ethoxy-1,6-diphenyl-4-thio-2,3,5-triazaine(H_2MacL^1) in ethanol reacts with chromium chloride, the macrocyclic ligand H_2MacL^2 is formed. The complexes have been characterized on the basis of elemental analysis, molar conductance, magnetic susceptibility, IR, ^1H NMR, ^{119}Sn NMR, ^{207}Pb NMR and X-ray spectral studies. The newly designed products were tested for their antimicrobial activity. Histopathological studies of rat livers treated with the test compounds were also carried out to ascertain the activity. All the results are discussed with significant bioresponces.

INTRODUCTION

Schiff bases constitute one of the most important class of the biologically active ligands, providing potential binding sites through nitrogen and sulfur-oxygen donor atoms. The ease of formation of a variety of metal complexes from these ligands speaks for their spectacular progress in coordination and bioinorganic chemistry. The chemistry of the Schiff base complexes attracts many researches /1-4/ because of its wide applications in food industry, dyes industry, analytical chemistry, catalysis, fungicides and in agrochemical fields. The innovative work of the Schiff bases and their metal complexes plays a key role in our understanding of the coordination chemistry of metal ions /5,6/. Schiff base macrocyclic ligands derived from thiosemicarbazide are of significant interest not only for their pharmacological properties as antibacterial,

* Author for correspondence: Professor R.V. Singh, Department of Chemistry, University of Rajasthan, Jaipur – 302 004, INDIA, E-mail : kudiwal@datainfosys.net; Fax : +91-141-2708621

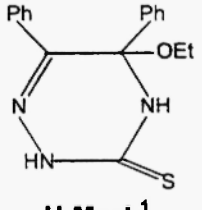
anticancer, antiviral and antifungal agents /7,8/, but also for their capacity for chemical recognition of anions and metals of biochemical, medical and environmental importance /9,10/. They can yield mono- or polynuclear complexes, some of which are biologically relevant /11,12/. In particular, main group metal complexes with such ligands containing a thiosemicarbazone moiety have a wide range of biological properties /13/. The number and relative proportion of donor atoms, and the cavity size in the macrocyclic compounds, give special reactivity to these molecules. The most important factors in the condensation /14/ reactions[1+1], [1+2] or [2+2], causing the relative proportions these reactions, are : chain length and presence of heteroatoms in the precursor or molecules, type of condensation, experimental conditions such as solvent, pH and temperature. In connection with previous investigations /15-17/ on the coordinating properties of lead(II) and tin(II), to isolate new macrocyclic complexes with potential antimicrobial properties, we have focussed on the synthesis, spectroscopic and biochemical aspects of lead and tin complexes of thiosemicarbazone moiety.

EXPERIMENTAL

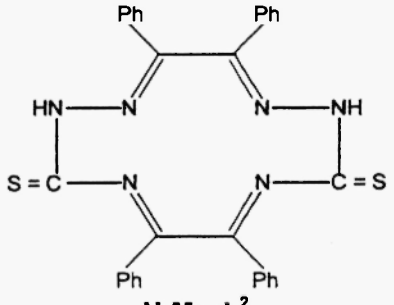
All the chemicals used were of Anala R grade. The solvents were purified according to the standard procedures before use.

Synthesis of the Ligand H_2MacL^1 :

An ethanolic solution (30 mL) of thiosemicarbazide (0.01 mol, 0.92 g) was added to an ethanolic (30 mL) solution of benzil (0.01 mol, 2.1 g) in the presence of 15 mL of 2M HCl. The reagents were added alternatively dropwise with stirring at room temperature. After addition of the reagents, the mixture was heated with stirring for 7-10 h. On keeping it overnight, a yellow-coloured crystalline solid was formed, which was filtered, washed with ethanol and dried *in vacuo*.



H_2MacL^1



H_2MacL^2

Synthesis of the Macrocyclic Ligand H_2MacL^2 :

An ethanolic solution (25 mL) of chromium (III) chloride hydrate (0.001 mol, 0.26g) was added to an ethanolic solution (25 mL) of H_2MacL^1 (0.001 mol, 0.31g). The mixture was stirred at room temperature

until the cream-coloured solid of H_2MacL^2 formed, which was filtered, washed with ethanol and dried *in vacuo*.

Synthesis of the Complexes :

A warm ethanolic (10 mL) solution of corresponding metal salt (0.001 mol) was added to a warm ethanolic (10 mL) suspension of H_2L^2 (0.001 mol, 0.55 g). The mixture was heated under reflux with stirring for 5h. On cooling a coloured complex precipited out, which was filtered, washed with ethanol and dried *in vacuo*.

The reaction of a H_2MacL^1 with chromium chloride in ethanol at room temperature yields a cream-coloured solid of H_2MacL^2, i.e., a macrocyclic ligand. The reaction of macrocycle H_2MacL^1 with chromium under the described conditions is the first direct procedure to isolate a free macrocycle H_2MacL^2 containing a thiosemicarbazone moiety. The complexes are stable in air. The analytical data of the complexes are consistent with the proposed stoichiometries, which are summarized in Table 1.

Table 1

Physical Properties and Analytical Data of the Ligands and their Metal Complexes

Compound/ Molecular Formula	M.P.°C and Colour	Yield (%)	Analysis, Found (Calcd.) %				Mol. Wt. Found (Calcd.)
			C	H	N	M	
H_2MacL^1 $C_{17}H_{17}N_3OS$	190 Yellow	68	65.25 (65.57)	5.25 (5.51)	13.24 (13.49)	-	288 (311)
H_2MacL^2 $C_{30}H_{22}N_6S_2$	170 Cream	66	67.65 (67.90)	3.99 (4.18)	15.65 (15.84)	-	506 (531)
$[Sn(H_2MacL^2)Cl_2]$ (1)	205 White	50	50.70 (50.05)	2.88 (3.08)	10.47 (11.67)	15.93 (16.48)	688 (720)
$[Pb(H_2MacL^2)Cl_2]$ (2)	198 White	57	44.38 (44.54)	2.56 (2.74)	9.06 (10.38)	25.03 (25.58)	784 (809)
$[Pb(H_2MacL^2)(NO_3)_2]$ (3)	212 White	59	41.59 (41.80)	2.34 (2.57)	11.35 (13.00)	23.44 24.01)	833 (862)
$[Pb(H_2MacL^2)(CH_3COO)_2]$ (4)	227 White	62	47.68 (47.71)	3.66 (3.30)	9.27 (9.82)	24.68 (24.21)	825 (856)

Physical Measurements

The molecular weights were determined by the Rast Camphor Method. Conductivity measurements in dry dimethylformamide were performed with a conductivity bridge type 305. Infrared spectra were recorded as KBr discs on a Nicolet Magna FT-IR 550 spectrophotometer. ^1H NMR spectra were recorded on a FX 90Q spectrometer in DMSO-d_6, using TMS as the internal standard. The X-ray powder diffraction measurements

were performed on a Philip X-ray diffractometer (model PW 1840), having Fe-Kα target and operated at 30 Kb and 40mA. The intensity of Fe-Kα radiation diffracted from the powder specimen was detected by a solid state detector and recorded as function of 2θ. Nitrogen and chlorine were estimated by Kjeldahl's and Volhard's method, respectively. Tin was estimated as SnO_2 and lead was estimated as lead sulphate gravimetrically.

RESULTS AND DISCUSSION

Mass Spectra

The mass spectrum of H_2MacL^1 confirms the proposed formula showing a peak at 311 amu corresponding to the molecular ion $[C_{17}H_{17}N_3OS]^+$. It also shows a series of peaks corresponding to the loss of ethanol, i.e., at 266 amu ($M-C_2H_5OH^+$) and various fragments. These data suggest the [1+1] cyclization of benzil and thiosemicarbazide. The mass spectrum of H_2MacL^2 shows a peak at 530 amu corresponding to the macrocyclic species $[C_{30}H_{22}N_6S_2]^+$. It also shows a series of peaks corresponding to various fragments. Their intensity gives the idea of the stability of the fragments.

I.R. Spectra

The infrared spectra of the ligand and its complexes give important information regarding the coordination to the metal ion. The absence of the bands in the region 2600-2800 cm^{-1} and its metal complexes suggest the absence of any thiol tautomer in the solid state /18/.

The IR spectrum of H_2MacL^2 shows several bands in the region 3390-3070 cm^{-1} corresponding to N-H stretching vibrations, which indicates that the ligand is present in neutral form according to the analytical data. The most significant bands of H_2MacL^2 are: 3249, 3160 cm^{-1} (N-H), 1615 cm^{-1} (C=N), 1480 cm^{-1} (thioamide I) and 760 cm^{-1} (thioamide II).

The IR spectra of lead(II) and tin(II) complexes show the C=N band slightly shifting to higher frequency, due to bond formation to the metal ion through four imine nitrogen atoms. The bands assigned to thioamide remain at the same position as in the free ligand, which indicates that this group is not involved in coordination /19/. The spectra of the nitrato complexes display a broad absorption band at 1390 cm^{-1}, which corresponds to the coordinated nitrate group.

^{1}H NMR Spectra

^{1}H NMR spectral data of H_2MacL^1 in $CDCl_3$ confirm the absence of terminal amine group and the presence of inserted ethanol, as well as two signals assigned to the N-H group. Signals are exhibited at ppm δ 10.3s (NH), 10.8s (NH), 7.40 – 7.21m (Ph), 3.4q (CH_2) and 1.4t (CH_3).

The ^{1}HNMR spectrum of H_2MacL^2 confirms the absence of ethanol, either inserted or as a crystallization molecule. It exhibits signals at δ ppm as: 10.6s(NH), 11.3s(NH) and 7.45-8.16m(Ph).

^{119}Sn NMR Spectra

The ^{119}Sn NMR spectra of the tin complexes give signals at -δ585-596 ppm, indicating coordination number six around the tin atom in the complexes /20/.

^{207}Pb NMR Spectra

The ^{207}Pb NMR spectra have proved to be a powerful tool in assessing the coordination number of lead and in turn elucidating the structures of the derivatives. The ^{207}Pb nuclear magnetic resonance spectra of the lead complexes exhibited a singlet at -δ1955-1969 ppm, indicating the hexacoordinated state.

X-Ray Diffraction Studies

The X-ray diffraction studies of the finely powdered sample of the compound $[Sn(H_2MacL^2)Cl_2]$ have been carried out in order to have an idea about the lattice dynamics of the compound. The observed interplanar spacing values ('d' in A°), have been measured from the diffractogram of the compound and Miller indices h, k and l have been assigned to each d value and 2θ angles are reported in Table 2. The results show that the compound belongs to the 'orthorhombic' crystal system, having unit cell parameters:

a = 25.775 b = 17.542 c = 10.297

max. dev of 2θ = 0.1

α = β = γ = 90°C.

Table 2

X-Ray Powder Diffraction Data for the Compound $[Sn(H_2MacL^2)Cl_2]$

S.No.	2θ (obs.)	2θ (calcd.)	Delta	h	k	l	d-spacing (obs) Å
1	15.36	15.35	0.000	2	2	0	7.250
2	16.91	16.89	0.02	3	0	1	6.590
3	22.62	22.59	0.03	5	1	0	4.940
4	25.85	25.85	0.00	4	3	0	4.330
5	27.47	27.47	0.00	5	2	1	4.080
6	30.78	30.76	0.02	3	4	1	3.650
7	31.95	31.91	0.04	3	3	2	3.520
8	37.94	37.91	0.03	7	3	1	2.980
9	42.21	42.22	-0.00	4	3	3	2.690
10	44.65	44.65	0.00	10	1	0	2.550
11	46.38	46.38	-0.00	2	7	0	2.460
12	48.25	48.18	0.07	8	5	0	2.370

BIOLOGICAL STUDIES

Antifungal Activity

The antifungal activity has been evaluated against several fungi by the Radial Growth Method /21/. The compounds were directly mixed with the medium in 25, 50, 100 and 200 ppm concentrations. Controls were also run and three replicates were used in each case. The linear growth of the fungus was obtained by measuring the diameter of the fungal colony after 96 hours. The amount of growth inhibition in each of the replicates was calculated by the equation (δc - δt) x 100 / δc, where δc and δt are the diameters of the fungal colonies in the control plate and the test plate respectively.

Table 3

Fungicidal Screening Data of the Ligand and its Metal Complexes

Average (%) inhibition after 96 hours	Cone. in ppm	Compounds					Bavistin (Standard)
		H_2MacL^2	(1)	(2)	(3)	(4)	
Alternaria alternata	25	34	49	55	46	33	84
	50	45	60	76	65	43	87
	100	54	75	84	72	68	100
	200	61	100	100	100	79	100
Alternaria brassica	25	32	45	50	43	36	82
	50	42	56	68	56	48	91
	100	56	71	80	69	73	100
	200	60	100	95	75	88	100
Fusarium oxysporum	25	17	35	43.6	51	35	83
	50	26	48	53	66	47	86
	100	35	58	65	70	65	100
	200	44	69	74	78	74	100
Macrophomina phaseolina	25	19	38	45	40	31	82
	50	31	51	57	52	42	82
	100	39	63	69	66	53	100
	200	48	77	78	71	60	100

Antibacterial Activity :

The antibacterial activity of the synthesized compounds was determined *in vitro* using filter paper disc method /22/. Different species of gram positive and gram negative bacteria were used. The considered compounds were dissolved in 10% methanol, different concentrations have been chosen (125, 250, 500 μg/mL). Agar plates were inoculated uniformly from fresh broth culture of Gram +ve and Gram –ve bacteria.

The discs were incubated at 5 °C for 1h to permit good diffusion and then incubated at 28 °C for 24h. The formed zones were measured in mm. The results are shown in Table 4.

The results of Table 4 reveal that all the compounds are active against these organisms, even at low concentrations, and the inhibition of the growth of microorganism was found to be dependent on the concentration of the compounds. The results of the biological screening indicated that the metal chelates are more active than the starting materials and the ligand.

Table 4

Response of Various Microorganisms to Synthesiged Ligand and its Metal Complexes *in vitro* (Culture).

Compound	*Bacillus cereus*		*Bacillus subtilis*		*Escherichia coli*	
	A	Conc.	A	Conc.	A	Conc.
H_2MacL^2	+	125	+	125	+	250
$[Sn(H_2MacL^2)Cl_2]$	++	500	++	250	-	-
$\{Pb(H_2MacL^2)Cl_2]$	+++	125	++	250	++	500
$[Pb(H_2MacL^2)(NO_3)_2]$	+	250	+	500	++	125
$[Pb(H_2MacL^2)(CH_3COO)_2]$	++	500	+++	125	-	-
Sulphadiazine (Standard)	+++	125	+++	125	++	125

A = Antimicrobial activity of the tested compounds +, ++ and +++ represent the extent of the inhibition zones: (-) no inhibition was observed, i.e. compound is not active :

(+) = 1mm, slightly active

(++) = 2mm, moderately active

(+++) = (2-5mm), highly active

Concentration is calculated as μg / mL

Antihepatotoxic Activity

Treatment Schedule: Male albino rats of Sprague-Dawley strain were used in the present investigations. The rats were fed with balance pellet diet and water was provided *ad libitum*. They were divided into six groups containing six animals each. Animals of group A served as control and received only gum asacia (1%) in distilled water. Group B received (CCl_4(1.5 mL/kg b.w.) and gum asacia to produce hepatotoxicity. Groups C, D and E received H_2MacL^2, $[Sn(H_2MacL^2)Cl_2]$ and $[Pb(H_2MacL^2)Cl_2]$, respectively.

Assessment of Liver Function: The biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT) /23/, serum glutamic pyruvate transaminase (SGPT) /23/, alkaline phosphatase (ALKP) /24/, and total protein and total albumin /25,26/ were estimated by reported methods.

Histopathological Studies of Rat Liver: The histopathological studies were also carried out by the reported method /27/. The rats were sacrificed under light ether anasthesia after 24h of the last dosage, and then the livers were removed and washed with normal saline. Small pieces of liver tissues were processed and embeded in paraffin. Sections of 5-6 microns in thickness were cut, stained with haematoxylin eosin and then studied under an electron microscope.

The results of the biochemical estimations are reported as mean ± δ.E. Total variation presents in a set of data was estimated by one-way analysis of variance (ANOVA). Student 't' test and Dennett's test were used for determining the significance /28/.

Table 5

Effect of the Ligand and its Metal Complexes on Various Liver Enzymes of Albino Rats.

Group	Treatment	Mean ± SEM (units / ml)			Mean ± SEM	
		(SGOT) Serum glutamic oxaloacetic transaminase	(SGPT) Serum glutamic pyruvate transaminase	(ALKP) Alkaline phosphate	Total protein (TP)	Total albumin (TA)
A	Normal control	45.12±1.42	32.80±0.51	25.42±0.96	5.79±0.311	3.55±0.199
B	Toxic control	77.10±2.04	65.54±2.54	50.14±3.149	4.30±0.167	4.55±0.667
C	H_2MacL^2	50.02±1.58***	41.13±3.24***	18.24±1.25***	6.15±0.261***	4.25±0.11*
D	$[Sn(H_2MacL^2)Cl_2]$	47.64±2.45***	37.59±1.45***	21.96±2.08***	6.23±0.228***	3.75±0.144*
E	$[Pb(H_2MacL^2)Cl_2]$	35.98±1.40***	44.4±0.86***	21.89±3.58***	6.12±.260***	3.56±0.052
F	Silybon-70 (Standard)	56.76±1.56***	49.66±2.64*	26.19±1.417***	6.96±0.371***	3.66±0.59

*P < 0.05; *** P < 0.001

The values of biochemical parameters are in mean ± SEM.

Figures in parentheses indicate the % protection in individual biochemical parameters from their elevated values caused by the hepatotoxin.

CONCLUSION

The administration of carbon tetrachloride enhanced the levels of SGOT, SGPT and ALKP by 77.10, 65.54 and 50.14 units/mL in comparison to normal values of 45.12, 32.80 and 25.42 units/mL, respectively. The administration of pure isolated ligand and its complexes (15mg/kg b.w., p.o.) decreased the levels in the range 35.98 – 50.02 units / mL in the case of SGPT and 18.24 – 21.96 units/mL in the case of ALKP, which were found to be comparatively better than by the standard drug *Silybon* – 70 (56.76, 49.66 and 29.16 units/mL, respectively.

The toxicant also reduced the level of total protein (4.30 g/dl) and increased the level of total albumin (4.55 g/dl) in comparison to the normal values (5.79 g/dl and 3.55 g/dl, respectively). The administration of the isolated ligand and its metal complexes enhanced the reduced level of the total protein in the range 6.12 – 6.96 g/dl and decreased the elevated levels of total albumin in the range of 3.56 – 3.75 g/dl in comparison to the standard drug *Silybon* – 70 (6.96 and 3.66 g/dl, respectively).

The values of biochemical parameters are in mean ± SEM, n = 6 animals per group. Figures in parentheses indicate the % protection in individual biochemical parameters from their elevated values caused by the hepatotoxin.

The histopathological studies of the liver were also carried out, which showed swelling, necrosis in hepatocytes, fatty deposition and the classic view of degenerating liver in CCl_4-treated rats in comparison to normal control rats. Administration of different extracts and isolated pure compounds exhibited a significant recovery of hepatocytes in different sections of the liver. The ligand and its compounds exhibited significant recovery of the liver tissue, even better than the standard drug *Silybon*-70, wherein $[Pb(H_2MacL^2)Cl_2]$ showed prominent recovery of hepatocytes, disappearance of necrosis and fatty depositions and clearance of the central vein.

ACKNOWLEDGEMENT

The authors are grateful to Professor (Dr.) Mendel Zangen, Freund Publishing House Ltd, Tel Aviv, Israel for his kind suggestions in improving this manuscript.

REFERENCES

1. (a) D.W. Ward, C.D. Satish, V.H. Kulkarni and C.S. Bajgun, *Indian J. Chem.* **39A**, 2000, 415.
   (b) K.S. Ashok, G. Singh, K. Singh, K.N. Raj., N.H. Ram and S.N. Dubey, *Indian J. Chem.*, **38A**, 1999, 891.
2. S. Ozawa, Y. Watanabe, S. Nakashimo, T. Kitagawa and I. Morishima, *J. Am. Chem. Soc.*, **116**, 1994, 634.
3. P.J. Pessiki, S.V. Khangulov, D.M. Ho and G.C. Dismukes, *J. Am. Chem. Soc.*, **116**, 1994, 891.
4. K. Weighardt, *Angew. Chem. Int. Ed. Engl.*, **28**, 1990, 1153.
5. A.S. Goldstein, R.H. Beer and R.S. Drago, *J. Am. Chem. Soc.*, ***116***, 1994, 2424.
6. C.G. Young and A.G. Wedd, in: *Encyclopedia of Inorganic Chemistry*, R.B. King (Ed); Wiley, New York, 1994, 2330.
7. M. Wang, L.F. Wang, Y.Z. Li, Q.X. Li, Z.D. Xu and D.M. Qu, *Trans. Met. Chem.*, **26**, 2001, 307.
8. L.D.S. Yadav and S. Singh, *Indian J. Chem.*, **40B**, 2001, 440.
9. E. Labisbal, A. Sousa, A. Castineiras, A. Garcia-Vazquez, J. Romero and D.X. West, *Polyhedron*, **19**, 2000, 1255.
10. M.K. Srivastava, B. Mishra and M. Nizamuddin, *Indian J. Chem.*, **40B**, 2001, 342.
11. M.A. Ali, A.H. Mirza, A. Mansur, S. Hussain, M. Nizamuddin, *Polyhedron*, **20**, 2001, 1045.
12. F.M. Belicchi, F. Bisceglie and G. Pelosi, *J. Inorg. Biochem.*, **83**, 169 (2001).
13. E.M. Jouad, A. Riou, M. Allian, M.A. Khan and G.M. Bouet, *Polyhedron*, **20**, 2001, 67.
14. B. Dietrich, P. Viout and J.M. Lehn, *Macrocyclic Chemistry* (VCH, Weinheim) 1993.

15. A. Chaudhary and R.V. Singh, *Indian J. Chem.*, **40A**, 2001, 1130.
16. A. Chaudhary, S. Dave, R.K. Saini and R.V. Singh, *Main Group Met. Chem.*, **24**, 2001, 217.
17. A. Chaudhary, S. Dave, R. Swaroop and R.V. Singh, *Indian J. Chem.*, **40A**, 757 (2001).
18. D.E. Fenton and D.H. Cook, *J. Chem. Soc., Chem., Commun*, **1977**, 724.
19. K. Nakamoto, *Infrared and Raman Spectra of Coordination Compounds* #, Wiley Interscience, New York, 1970.
20. A. Chaudhary, R.V. Singh and A. Phor, *Indian J. Chem.*, **41A**, 2002, 2536.
21. A. Kumari, J.P. Tandon and R.V. Singh, *Appl. Organomet. Chem.*, 7, 1993, 655.
22. C. Leifert, H. Li, S. Chidburee, S. Hampson, S. Workman, D. Sigee, H.A.S. Epton and A. Harbour, *J. Appl. Bact.*, **78**, 1995, 97.
23. S. Reitman, S. Frankel, *Am. J. Clin. Pathal.*, **28**, 1957, 56.
24. O. Daniel, P.R.N. Kind and E.J.J. King, *J. Clin. Pathol.*, 7, 1954, 332.
25. I.D.P. Wootton, *Microanalysis in Medical Biochemistry*, 4th edn., A. Churchill Ltd., London, 1964; p. 138.
26. B.T. Dumas, W.A. Watson and H.G. Biggs, *Clin, Chim. Acta*, **31**, 1971, 87.
27. L.G. Luna, *Manual of Histology; Staining Methods of Armed Forces Institute of Pathology*, 3rd Edn., McGraw-Hill, Book Co., New York, 1968; p.1.