Synthesis, Spectroscopic Elucidation and Biomedical Applications of 1,10-Phenanthroline Derivatives of Lead(II)

Ashu Chaudhary and R.V. Singh*

Department of Chemistry, University of Rajasthan, Jaipur - 302 005

ABSTRACT

A huge world of inorganic drugs is waiting to be discovered. The inorganic drugs can be metal complexes wherein at least some of the ligands remain bound to the metal even on reaching the target site or where the ligands simply act as a vehicle for delivery and are not critical for activity. In some cases the ligands themselves may be the active species. A few inorganic compounds are already successful drugs. However, some more still needs to be done. The complexes can be formed in the body to handle dysfunction due to metal poisoning. Metal has the power to induce biological activity by interaction with DNA by thread binding mechanism. Achieving optimal chemopreventive potency with lowest toxicity continues to be our primary goal in designing and developing lead(II) complexes of phenenthrolines and dicarboxylic acids. These complexes have been characterized by chemical analysis, IR, ^{1}H NMR, ^{13}C NMR and ^{207}Pb NMR spectral studies, X-ray powder diffraction, molecular weight determinations and conductivity measurements. An octahedral geometry around the lead atom is suggested for $[Pb(MacL^{n})Cl_2]$ complexes. The pathogenicity of certain microbial infections associated with the complexes made it obligatory to distinguish between parent counterparts and their lead(II) complexes. To make the subject interesting biomedical applications of the complexes will be discussed.

INTRODUCTION

The field of macrocyclic chemistry of metals is developing very fast because of its variety of applications and importance in the area of coordination chemistry /1/. There has been a spectacular growth in the interest in metal complexes with tetraazamacrocyclic ligands, followed by the extensive work on metal control template synthesis of macrocyclic species /2,3/. Macrocyclic complexes have been synthesized with a rational approach which possess a broader spectrum of antitumour activities, low toxicity and lack of cross resistance. By more frequent use of the combinations of organic plus inorganic drugs, as well as monitoring

* 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

the effects of various drugs on the distribution elements, the therapy of various diseases can become more sophisticated.

The complexes of 1,10-phenanthrolines are at the forefront of bioinorganic chemistry due to their bacteriostatic and bacteriocidal properties /4/. 1,10-Phenanthroline (1,10-phen), 2,2′-bipyridine (2,2′-bipy) and their substituted derivatives, both in the metal-free state and as ligands coordinated to transition metals, disturb the functioning of a wide variety of biological systems /5/. However, m- and p-substituted phenanthrolines were less effective than 1,10-phenanthroline at preventing fungal growth /6/ and 2,9-dimethyl-1,10-phenanthroline was the most potent inhibitor /7/. Extensive microbiological and pharmacological investigations with phenanthrolines and related chelates led to the clinical studies with the highly stable Ni(II) complexes of 3,4,7,8-tetramethyl-1,10-phenanthroline /8/. These complexes were known to have a wide spectrum of antimicrobial actions, and to produce negligible toxicity to skin and mucous membrane /9/. Lead complexes are used in the treatment, management or diagnosis of disease. Therefore, we have designed and developed lead(II) complexes with macrocyclic ligands (MacL) consisting of 1,10-phenanthroline with dicarboxylic acids. Antimicrobial and antifertility activity of the complexes has also been discussed.

EXPERIMENTAL

All solvents used were of high purity and distilled before use, $PbCl_2$ (BDH), malonic acid, succinic acid, glutaric acid and adipic acid (Fluka) and 1,10-phenanthroline (E. Merck) were used as obtained.

Synthesis of the ligand ($MacL^1$)

In a 100 mL short necked round bottom flask, a weighed amount of 1,10-phenthroline was taken and to this a corresponding amount of malonic acid in methanol was added. The reaction was carried out in 2:2 molar ratios and heated under reflux for 12 hours. The reaction mixture was cooled and the off-white compound obtained was recrystallized from methanol.

The same procedure has been used for the syntheses of $MacL^2$, $MacL^3$ and $MacL^4$. The reagents used were succinic acid, glutaric acid and adipic acid, respectively, in place of malonic acid. The physical properties and analytical data of the ligands are given in Table 1.

Synthesis of the complex [$Pb(MacL^1)Cl_2$]

The reaction mixture containing $MacL^1$ and $PbCl_2$ in 1:1 molar ratio in methanol was heated under reflux for 42 hours. The reaction mixture was cooled, transferred to an evaporating dish and set aside for a few hours, whereupon a white compound separated out. The product formed was washed and dried under reduced pressure, and recrystallized from a 1:1 mixture of toluene and n-hexane. The same procedure has been used for the syntheses of [$Pb(MacL^2)Cl_2$], [$Pb(MacL^2)Cl_2$] and [$Pb(MacL^4)Cl_2$] using $MacL^2$, $MacL^3$ and $MacL^4$ as ligands, respectively.

Physical Measurements

The molecular weights were determined by the Rast Camphor method. Conductivity measurements in dry dimethylformamide were performed with a conductivity bridge type 305. Infrared spectra were recorded on a Nicolet Magna FT-IR 550 spectrophotometer in KBr pellets. ^{1}H NMR spectra were recorded on a JEOL FX-90Q spectrometer in $CDCl_3$ using TMS as the internal standard. ^{13}C and ^{207}Pb NMR spectra were also recorded on the same spectrometer using MeOH as the solvent at 22.49 MHz and 33.35 MHz, respectively. Nitrogen and chlorine were estimated by Kjeldahl's and Volhard's method, respectively. Lead was estimated as lead oxide gravimetrically. Carbon and hydrogen analyses were performed at Central Drugs Research Institute (CDRI) Lucknow. The physical properties and analytical data of the metal complexes are also listed in Table 1.

Table 1

Physical Properties and Analytical Data of the Ligands and their Lead Complexes.

Compound	M.P. °C and Colour	Analysis, Found (Calcd.) %					Mol. Wt. Found (Calcd.)
		C	H	N	Cl	Pb	
$MacL^1$	180 Off white	72.48 (72.58)	3.15 (3.25)	10.49 (11.28)	-	-	473 (496.48)
$MacL^2$	189 Off white	73.07 (73.27)	(3.73 (3.84)	9.83 (10.68)	-	-	(524.54)
$MacL^3$	177 Off white	73.80 (73.90)	4.26 (4.38)	9.33 (10.14)	-	-	495 (552.59)
$MacL^4$	198 Off white	74.27 (84.47)	6.25 (6.25)	8.82 (9.65)	-	-	548 (580.64)
$[Pb(MacL^1)Cl_2]$	232 White	46.22 (46.52)	2.04 (2.08)	6.39 (7.23)	8.59 (9.15)	26.30 (26.72)	753 (774.59)
$[Pb(MacL^2)Cl_2]$	235 Light yellow	47.69 (47.89)	2.41 (2.51)	6.23 (6.98)	8.31 (8.83)	25.30 (25.79)	778 (802.64)
$[Pb(MacL^3)Cl_2]$	210 Light yellow	49.00 (49.16)	2.70 (2.91)	5.96 (6.74)	8.02 (8.54)	24.50 (24.92)	804 (830.70)
$[Pb(MacL^4)Cl_2]$	217 White	50.00 (50.35)	3.19 (3.29)	5.71 (6.52)	7.68 (8.26)	23.64 (24.10)	829 (858.75)

RESULTS AND DISCUSSION

The products formed are solids and soluble in methanol, benzene, carbontetrachloride,

dimethylsulphoxide and dimethyl formamide. The conductivity values measured for 10^{-3}M solutions in anhydrous DMF are in the range 17-29 ohm^{-1} cm^2 mol^{-1}, showing them to be non-electrolytes.

IR spectra

The IR spectra of the starting materials and their lead complexes were recorded and their comparative studies confirmed the formation of macrocyclic complexes with the proposed coordination pattern /10,11/. The significant band in the compounds observed at 1600 - 1610cm^{-1} is assigned to the (C=N) vibration. This suggests bidentate (NN) coordination of phenanthroline in the compounds. This has been further confirmed by the appearance of new bands at 380-449 cm^{-1} in the spectra of the complexes. This band is due to Pb←N bond. Bands in the region 240 - 293 cm^{-1} are due to Pb-Cl bond.

^{1}H NMR spectra

^{1}H NMR spectra do not show any signal corresponding to the hydroxyl group. Proton signals due to 1,10-phenanthroline appear at δ 7.41 – 9.17 ppm. In the spectra of the complexes, a singlet appearing in the regions δ 2.90 – 2.96 and δ 3.15 – 3.19 ppm is assigned to methylene protons of malonic and succinic acid respectively, while a multiplet observed in the regions δ 3.21 – 3.24 and δ 3.28 – 3.30 ppm is ascribed to the methylene protons of glutaric acid and adipic acid respectively (Table 2).

Table 2

^{1}H NMR Spectral Data of the Ligands and their Lead(II) Complexes.

Complexes	Phenanthroline moiety	CH_2	$-(CH_2)_2-$	$-(CH_2)_3$	$-(CH_2)_4$
$MacL^1$	7.48 – 9.05	2.90	-	-	-
$MacL^2$	7.41 – 9.17	-	3.15	-	-
$MacL^3$	7.45 – 9.10	-	-	3.21	-
$MacL^4$	7.46 – 9.12	-	-	-	3.28
$[Pb(MacL^1)Cl_2]$	7.53 – 9.15	2.96	-	-	-
$[Pb(MacL^2)Cl_2]$	7.55 – 9.16	-	3.19	-	-
$[Pb(MacL^3)Cl_2]$	8.50 – 9.10	-	-	3.24	-
$[Pb(MacL^4)Cl_2]$	7.41-9.10	-	-	-	3.30

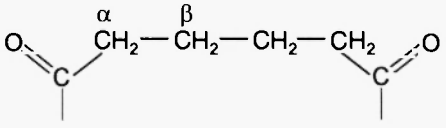
^{13}C NMR spectra

The conclusions drawn from the IR and ^{1}H NMR spectra are in agreement with the ^{13}C NMR spectral data (Table 3) regarding the authenticity of the proposed structure.

Table 3

^{13}C NMR Spectral Data of the Ligands and their Lead(II) Complexes.

Compound	C=O	Phenanthroline moiety	C_α	C_β
$MacL^1$	176.52	C_2 136.6; C_3 126.5; C_4 123.4; C_5 128.4; C_6 149.9	33.91	-
$MacL^2$	177.84	C_2 134.2; C_3 124.3; C_4 121.0; C_5 127.1; C_6 148.8	33.62	-
$MacL^3$	175.92	C_2 138.4; C_3 125.2; C_4 122.8; C_5 128.2; C_6 148.9	33.98	26.22
$MacL^4$	177.66	C_2 135.8; C_3 125.4; C_4 124.2; C_5 129.2; C_6 150.4	33.27	25.28
$[Pb(MacL^1)Cl_2]$	175.86	C_2 136.8; C_3 125.8; C_4 123.6; C_5 127.9; C_6 149.6	32.80	-
$[Pb(MacL^2)Cl_2]$	176.80	C_2 138.4; C_3 125.2; C_4 122.4; C_5 128.3; C_6 148.2	32.52	-
$[Pb(MacL^3)Cl_2]$	176.24	C_2 135.6; C_3 124.3; C_4 121.2; C_5 129.1; C_6 148.8	33.70	23.28
$[Pb(MacL^4)Cl_2]$	177.10	C_2 138.4; C_3 126.4; C_4 123.4; C_5 129.0; C_6 149.2	33.56	25.82



X-Ray

The X-ray powder diffraction study of the compound $[Pb(MacL^4)Cl_2]$ has been carried out in order to have an idea about the lattice dynamics of the compound. The results (Table 4) show that the compound belongs to the "orthorhombic" crystal system having unit cell parameters a = 32.8216, b = 12.8864, c = 19.8252, $\alpha = \beta = \gamma = 90^o$.

Table 4

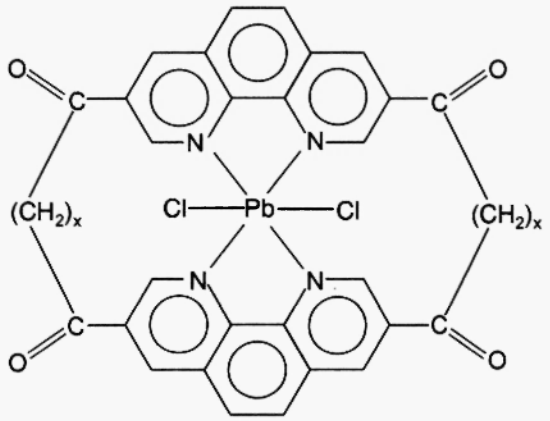
X-ray Powder Diffraction Data of $[Pb(MacL^1)Cl_2]$

Peak No.	20 (Calcd.)	2θ (deg.) (obs.)	d-spacing (obs.) (A°)	h	k	l
1	17.90	18.04	6.227	0	2	1
2	19.10	19.29	5.839	2	2	1
3	26.10	25.97	4.290	7	1	1
4	28.60	28.43	3.922	0	1	2
5	34.70	34.76	3.248	10	0	1
6	34.70	34.70	3.248	1	3	4
7	42.50	42.34	2.673	1	2	2

^{27}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 -δ 1950 – 1965 ppm, indicating the hexacoordinated state /12/.

On the basis of the above results the structure shown in Fig. 1 is proposed for the complexes.



where x = 1, 2, 3 or 4

Fig. 1

Microbial Assays

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 respectively for the antibacterial and antifungal activities.

The target microorganisms included *Aspergillus niger* NCIM 545, *Candida albicans* NCIM 3471, *X. campestris*, *Pseudomonas aeruginosa* NCIM 2036 and *Staphylococcus aureous* NCIM 2054 . These strains were selected because they are routinely used in testing of disinfectants /13/. The stock cultures of these microorganisms were maintained at 20°C in 15% glycerol /14/. The inoculum was prepared from stock cultures by 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 test tubes and sterilized in the autoclave at 121°C for 15 minutes. The baths were then inoculated with respective cultures and incubated on an orbital shaker (150 ppm) overnight at 30°C A_{540} of bacterial cultures and candida albicans was adjusted to 0.12 and 0.20 respectively. This corresponds to 10^6-10^7 colony forming unit (Cfu/ml). The spore inoculum of *A. niger* containing 10^6 spores per ml was used. The solutions of the ligands and complexes were prepared in DMSO /15/ and added to the tubes containing 3 ml liquid medium and inoculated with 30 µl of the cultures. Incubation was done for 18 h at 37°C. The extent of inhibition of *Staphylococcus aureus* by the ligands is found to be more pronounced than by their respective complexes, but the reverse is found for the other microorganisms. The experimental data of these complexes are listed in Table 5.

Table 5

Antimicrobial Activity* of the Ligands and their Lead(II) Complexes

Compound	*A. niger*	*C. albicans*	*X. campestris*	*P. aeruginosa*	*S. aureus*
Diclone (Standard)	8	16	8	9	15
$MacL^1$	7.3	8.9	9.5	10.3	9
$MacL^2$	8.0	9.6	10.3	-	12
$MacL^3$	7.5	10.2	-	11.7	12
$MacL^4$	8.0	14.7	11.0	12.2	-
$[Pb(MacL^1)Cl_2]$	9.2	18	13.2	12.0	15.5
$[Pb(MacL^2)Cl_2]$	10.5	17.7	14.0	18.3	16.3
$[Pb(MacL^3)Cl_2]$	12.4	20	15.1	-	17.1
$[Pb(MacL^4)Cl_2]$	14.6	22	16.0	19.1	19.8

*Zone of inhibition in mm.

Antifertility Activity

Experiments were conducted *in vivo* in male rats to check the antifertility activity. Compounds that showed good antimicrobial activity were chosen for the antifertility tests on male rats. The antifertility activity in male rats was carried out by using the following method. Twenty five adult male rats (body weight 40 – 50 g) were divided into five groups of five animals each. The animals were maintained and fed with

balanced pellet diet and tap water was provided *ad libitum*. One group was used as a control, and each animal of this group received 0.2 cm^3 olive oil per day orally. The ligands and their complexes were suspended in olive oil separately and given to animals orally at a dose level of 20 mg day^{-1} per kilogram body weight for 60 days. At 24 h after the last administration of the compound, the animals were autopsied and the reproductive tract was dissected out and the motility and the sperm count were measured.

The results reported in Table 6 reveal that there is a significant decrease ($P< 0.01$) in the motility from 78.5 ± 3.7 to 40.0 ± 5.2 in animals treated with the ligands. The sperm density also decreased ($P<0.001$) from 4.3 ± 0.42 to 2.4 ± 0.15 in testes and from 61.0 ± 4.8 to 42.5 ± 4.2 in cauda epididymis. A highly significant ($P < 0.001$) decline in the motility of sperm was observed in the case of lead complexes as compared to the ligands. Thus, it can be postulated that chelation through the ligand induces sterilizing activity in the biological systems.

Table 6

Effects of the Ligands and their Lead(II) Complexes on Sperm Dynamics and Fertility of Male Rats (Values are Expressed as Mean Plus / Minus SE)

Compound	Sperm motility (cauda epididymis) %	Sperm density		Fertility Test
		Testes	Cauda epididymis	
Vehicle alone (Olive oil)	7.85 ± 3.7	4.3 ± 0.42	61.1 ± 4.8	100% positive
$MacL^3$	47.5 ± 3.5[b]	2.9 ± 0.10[b]	50.5 ± 3.4[b]	80% negative
$MacL^4$	40.0 ± 5.2[c]	2.4 ± 0.15[c]	42.5 ± 4.2[c]	83% negative
$[Sn(MacL^3)Cl_2]$	35.0 ± 3.6[b]	1.2 ± 0.14	30.9 ± 4.3	92% negative
$[Sn(MacL^4)Cl_2]$	33.0 ± 4.2[a]	1.2 ± 0.12[c]	30.0 ± 3.0[c]	95% negative

[a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.02$

ACKNOWLEDGEMENT

One of the authors (Ashu Chaudhary) is indebted to CSIR New Delhi for financial assistance in the form RA vide grant No. 9/149/(374)/2K2 EMR/I/2004.

REFERENCES

1. S. Chandra and R. Singh, *Indian J. Chem.*, **34A**, 1003 (1995).
2. I.M. Atkinson, P.J. Baillie, N. Choi, F. Fabbrizzi, L.F. Lindoy, M.Mc. Partlin and P.A. Tasker, *J. Chem., Soc., Dalton Trans,* **1996**, 3045.
3. A. Campos, J.R. Anacona and M.M. Campos-Vallette, *Main Group Met. Chem.*, **22,** 283 (1999).

4. A.D. Krik and H.U. Gudel, *Inorg. Chem.*, **31**, 4564 (1992).
5. M.T. Reetz, E. Bohres and R. Goddard, *J. Chem. Soc. Chem. Commun*, **1998**, 935.
6. R.A. August, J.A. Khan, C.N. Moody and D.W. Young. *J. Chem. Soc. Perkin Trans. I.*, **1996**, 507.
7. W.W. Brandt, F.P. Dwyer and E.C. Gyarfas, *Chem. Rev.*, **54**, 959 (1954).
8. R.J. Rao and H.B. Wankhade, *Main Group Met. Chem.*, **19**, 239 (1996).
9. C. Platas, F. Avecilla, A. De Blas, T.Rodriguez-Blas and J.C.G. Bunzli, *J. Chem. Soc. Dalton Trans.*, **1999**, 1763.
10. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, *54th edn., Part B*, Wiley, New York, 1997.
11. M. Geraghty, M. Mc Cann, M. Devereux, F. Cronin, M. Curran and V. Mc Kee, *Metal-Based Drugs*, **6**, 41 (1999).
12. A. Chaudhary, A. Phor and R.V. Singh, *Main Group Met. Chem.* **27**, 1 (2004)
13. W.A. Olsen, *Aust. J. Hort. Pharm.*, **9**, 127 (1979).
14. D. Jones, P.A. Dell and P.H.A. Sneath, *A. Manual of Laboratory Methods*, Academic Press, 1984; 35
15. J.F. Ryley and W.G. Rathmell, *Symposium of British Mycological Society*, **63**, 1984.