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Investigations on pharmaceutically important 14-20-membered macrocyclic complexes : Designing, synthesis (*Via green tool*), spectroscopic characterization, antifungal, antibacterial and antifertility activity

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ABSTRACT

A huge world of inorganic drugs is waiting to be discovered. The inorganic drugs can be metal complexes wherein 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 or 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. Achieving optimal chemopreventive potency with lowest toxicity continues to be our primary goal in designing and developing Iron (II) and Lead (II) complexes. Tetraazamacrocyclic complexes of Iron (II) and Lead (II) have been synthesized by template condensation of $FeCl_2.4H_2O$ and $PbCl_2$ with dicarboxylic acid (malonic, succinic, glutaric or adipic acid) and 9,10-diaminophenantherene in 1:2:2 molar ratios. The new products with octahedral geometry have been characterized by elemental analyses, molecular weight determinations, molar conductance, magnetic moment and spectral studies *viz.*, infrared and electronic. On the basis of the spectral studies the binding sites are proposed as the nitrogen atom of the macrocycles. The formulation of the complexes as $[M(Mac^n)Cl_2]$ (where M= Fe or Pb and n=1 - 4) has been established on the basis of chemical composition. To assess the growth inhibiting potential of the iron (II) and lead complexes biological screening have been undertaken. Particular attention has been paid to the antifertility activity. The testicular sperm density sperm morphology, sperm motility, density of cauda epididymis, spermatozoa and fertility in mating trial and biochemical parameters of the reproductive organs of the male albino rats were examined.

KEYWORDS

Iron(II) complexes;
Lead(II) complexes
macrocyclic;
Spectral studies;
Bioactivity.

INTRODUCTION

Traditionally, synthesis of molecules involves use of chemicals, which are hazardous and put at risk both human beings and environment. Green chemistry as applied to chemical processes can be environmentally benign (in terms of reduction of energy, auxillaries, waste etc) and should always lead to simplification of pro-

cesses in terms of chemicals used and steps involved. Recently, the acceleration of a wide range of chemical reactions using microwave dielectric heating has been reported[1]. This in situ mode of energy conversion has many attractions to the chemist, because its magnitude depends on the properties of the molecules[2]. Studies of new types of chemotherapeutically important macrocyclic ligands and metal coordinated drugs are now attracting much attention[3].The multifarious role of transition metals in biochemistry[4,5] suggested that consideration potential exists for the development of new chemistries with these metals in ligand systems specifically designed to serve these roles. The enormous interest in the synthesis of the transition metal complexes of the nitrogen donor ligands arises due to the wide range of pharmacological activities of these compounds, which in several cases are known to have been enhanced by the presence of transition metals[6,7]. Macrocyclic ligands display a number of features of chemical interest. Research into the synthesis, structure and properties of transition metal macrocyclic complexes to model a wide variety of metalloprotein active sites or to mimic their chemistry is well established[8,9]. Macrocycles have wide applications in medicine, cancer diagnosis[10] and in the treatment of tumors[11]. A variety of lead complexes have also been reported to possess fungicidal as well as bactericidal activities[12-14] . Thiophenyl tri-phenyl-lead compounds possess anti-inflammatory property[15]. It has also been suggested that these are suitable for the treatment of various allergies asthma and influenza. Several organolead compounds find use as good algicides, herbicides and also as anticancerous agents[16-18].

Macrocyclic complexes of Pb(II) exhibit a broad spectrum of biological activity[19-21]. Further, it has been reported that iron salts (chloride, nitrate and acetate) cause loss of testicular germ cells in rats and rabbits and decreased libido and impotency were noted in men occupationally exposed to transition metal[22,23]. No work has been reported on Fe(II) and Pb(II) complexes with such type of tetraazamacrocyclic ligands. Therefore, the importance of the metal-nitrogen bonding and their prominence in agriculture, medicinal and industrial activity led us to synthesize and screen these compounds for their antifungal and antibacterial and antifertility activities. The complexes show good antifertility activity and may be useful for medicinal purpose.

EXPERIMENTAL

The chemicals include, malonic acid, succinic acid, glutaric acid and adipic acid (Fluka), 9,10-diamino phenentherene (E.Merck), $FeCl_2.4H_2O/PbCl_2$ (BDH).

Synthesis of the complexes [M (Mac^n)Cl_2]

(A) Traditional method

The reaction is carried out in 1:2:2 molar ratios. For the preparation of metal complexes, an ice cold solution of $FeCl_2.4H_2O/PbCl_2$ in methanol (50ml) was reacted with 9,10-diaminophenanthrene at 0°C and put in magnetically stirred 100ml round-bottom flask. This is followed by the addition of methanolic solution of malonic, succinic, glutaric or adipic acid). The reaction mixture was stirred continuously at room temperature for 10h. The resulting solid product was recovered by filtration, washed with methanol and dried in vacuo. These were recrystallized from a 1:1 solution of methanol and chloroform.

The purity of the compounds was checked by TLC on Silica Gel-G using anhydrous tetrahedrofuran as a solvent. Each of the compound moves as a single spot indicating the presence of only one component and hence their purity.

(B) Ecofriendly method

The unimolar and bimolar reaction mixture were taken in an open borosil beaker and irradiated inside a microwave oven until reaction gets completed. A drastic reduction in reaction time was thus observed due to the rapid heating capability of microwaves. The completion of the reaction was examined by TLC and the product was worked up as described in method A.

Analytical methods and physical measurements

Conductivity measurements of 10^{-3}M solutions were made with a Systronic Model 305 conductivity bridge in dry dimethylformamide at room temperature (28°C). Molecular weights were determined by the Rast Camphor Method. IR spectra were obtained as KBr pellets on a Perkin-Elmer 577 grating spectrophotometer in the range 4000-200 cm^{-1} and far IR spectra were also

recorded on the same spectrophotometer in Nujol Mulls using CsI cell. Electronic spectra in dimethylsulphoxide were recorded on a Hitachi W-2000 spectrophotometer. Magnetic moment of the complexes were determined by the Gouy's method at the room temperature. ^{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 33.35MHz., respectively. Nitrogen and chlorine were estimated by the Kjeldahl's and Volhard's method, respectively. The mass spectra of the complexes were recorded on a JEOL FX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 KV, 10 mA) as the FAB gas. Lead was estimated as lead oxide gravimetrically. Carbon and hydrogen analyses were performed at Central Drugs Research Institute (CDRI) Lucknow.

Biochemical procedure

(A) Antifungal activity

Antifungal activity of the complexes was studied on various fungi, namely Alternaria triticina (ATCC 698), Fusarium udum (ATCC 10245), Alternaria brassicae(ATCC 1050) Curvularia species(ATCC 12097), Helminthosporium oryzae(ATCC 11779), Aspergillus flavus, (ATCC 17884), Alternaria brasicicola(ATCC 29739) and Curvularia lunata(ATCC 10021) by using the spore germination technique[24,25].

Method

A drop of compound solution was placed on a grease-free glass slide and 50-100 spores of the test fungi were placed with the help of a sterilized inoculation needle on the solution. The slides were then placed in a moisture chamber and incubated at 25 $\pm$ 2°C, for 24 hours. After incubation, the spores were fixed and stained with lectophenol cotton blue, and spore germination was observed under a light microscope. Similar spore numbers of each fungus were mixed in sterilized distilled water, which served as control. For measurement of inhibition, the percentage germination was subtracted by a hundred to get percentage inhibition.

All the experiments were conducted in triplicate. The data were subjected to students 't' test for statistical significance.

Mycelial growth of five fungi, with or without chemicals, was observed by taking dry weight of fungi grown in 150ml conical flask. All the chemical flasks were filled with 50ml potato dextrose broth. Required amounts of the chemicals were then added to the broth to get the desired concentrations (100, 200 and 400 ppm) individually and in the mixture and dissolved and mixed thoroughly by shaking the flasks after autoclaving for 15 min. (at 121°C) the broth was allowed to cool down and 5mm disc of fungal mycelium was taken from the border of an actively growing fungal colony and incubated into the broth. The flasks were incubated at 25 $\pm$ 2°C for one week, Potato dextrose broth without the chemicals served as control. After one week, the broth with the fungal colony was determined by deducting the weight of the filter paper from the total weight of the filter paper and mycelium. All the experiments were conducted in triplicate. The data were subjected to student 't' test for statistical significance.

Statistical analysis

The data recorded for different concentrations of the compounds were subjected to the following statistical analysis.

Analysis of variance (ANOVA)

The analysis of variance was carried out separately for each fungus against all the compounds at various concentrations according to the procedure of Randomized Block Design Analysis[26].

(B) Antibacterial activity

In order to compare the antibacterial activity of iron(II) and lead(II) complexes, this activity was performed by paper disc plate method using Inhibition zone technique. The bacteria which were used in this method are namely *P.cepacicola* (ATCC 1079), *E.coli*, (ATCC 1920), *S.aureus* (ATCC 1450) and *K.aerogenous* (ATCC 793).

The activity against bacteria was evaluated by paper disc plate method (composition : peptone 5gm, beef extract 5 gm, NaCl 5 gm, agar-agar 20gm and distilled water 1 litre) was used for newly synthesized complexes and the reference drug used was streptomycin. All the compounds were dissolved in methanol in different concentrations. Paper discs of Whatman paper no.1; with a diameter of 5mm were soaked in these solutions.

These discs were placed on the medium previously seeded with the organisms in the Petri discs. These discs were stored in an incubator at 35 ± 2°C. The inhibition zone around each disc was measured (in mm) after 24-30 hours and activity index was calculated.

Activity index=Inhibition zone of the compound/Inhibition zone of the standard

Using inhibition zone technique, the antibacterial activity of the complexes were evaluated against the organisms P.cepaciola, Escherichia coli, Staphylococus aureus and K.aerogenous and their results have been enlisted in TABLE 9.

Antifertility activity

Experimental design

The rats were divided into 3 groups containing 6 animals in each

Groups	Treatment given
Group-I	Control or vehicle treated (0.5 ml olive oil)
Group-II	$[Fe(Mac^2)Cl_2]$ (5gm in 0.5 ml olive oil/day for 60 days
Group- III	$[Fe(Mac^4)Cl_2]$ (5gm in 0.5ml olive oil /day for 60 days)

The dose was administered orally and animals were weighed before and after the treatment.

Fertility test

The mating exposure test of control and treatment group were performed on day 55 using the method of WHO (W.H.O protocol 1983). The mated females were separated and the implantation sites were recorded on the 16th day of pregnancy through laparotomy , number and weight of litters were recorded.

Autopsy

The animals were weighed and autopsied under light ether anesthesia 24 hours after the last dose of the treatment.

Body and organ weight measurements

Initial and final body weights of the animals were recorded. Autopsy the reproductive and accessory sex organs (Testes, epididymus, seminal vesicle, ventral prostate and vasa deferens) along with liver were dissected out, freed from adherent tissues and weighed up to the nearest 0.01 gm.

Sperm motility and sperm density[27,28]

After anesthesizing the rats the epididymus was exposed by scrotal incision, and spermatozoa were expressed out by cutting the distal end of the cauda epididymal tubule spermatozoa with epididymal fluid was diluted with physiological saline and placed on a thin glass slide and forwarded motility of 100 spermatozoa per rat was observed under microscope using pre-calibrated micrometer .

Spermatozoa were counted by placing the sperm suspension on both sides of Neubauers hemocytometer and allowed to settle in a humid chamber for 1 hour. The number of spermatozoa in the appropriate squares of hemocytometer was calculated under the microscope at 100 X magnification.

Hematology[29]

Blood was collected through cardiac puncture and the values of RBC and counts, haematocrit, haemoglobin%, MCV, MCH and MCHC were calculated.

RESULTS AND DISCUSSION

The physical properties and analytical data of the complexes are given in TABLE 1. All the complexes are slightly soluble in common organic solvents but highly soluble in DMF and DMSO. The molecular conductance in anhydrous DMF are in the range 15-29 ohm^{-1} cm^2 mol^{-1} showing them to be non-electrolytes. Mo-

TABLE 1: Physical properties and analytical data of the Iron(II) and Lead(II) macrocyclic complexes

Compound	M.p. °C and colour	Analysis, Found (Calcd.) %					Mol. Wt. found(Calcd.)
		C	H	N	Cl	M	
$[Fe(Mac^1)Cl_2]$,	166 Brown	60.00 (60.10)	3.46 (3.56)	7.48(8.25)	10.02(10.44)	7.73(8.22)	656(679.44)
$[Fe(Mac^2)Cl_2]$,	178 Brown	61.00(61.12)	3.79 (3.99)	7.13(7.92)	9.56(10.02)	7.44(7.89)	688(707.50)
$[Fe(Mac^3)Cl_2]$,	154 Brown	62.00(62.05)	4.30 (4.38)	6.90(7.62)	9.18(9.64)	7.12(7.59)	711(735.55)
$[Fe(Mac^4)Cl_2]$,	158 Brown	62.70(62.91)	4.70 (4.75)	6.55(7.34)	8.84(9.29)	6.86(7.31)	741(763.61)
$[Pb(Mac^1)Cl_2]$,	218 White	49.0(49.16)	2.70(2.91)	5.94(6.74)	8.14(8.54)	24.49(24.91)	803(830.75)
$[Pb(Mac^2)Cl_2]$,	207 White	50.10(50.35)	3.18(3.29)	5.81(6.52)	7.80(8.26)	23.65(24.10)	836(858.80)
$[Pb(Mac^3)Cl_2]$,	210 White	51.30(51.46)	3.58(3.64)	5.56(6.32)	7.52(8.00)	22.92(23.34)	867(886.86)
$[Pb(Mac^4)Cl_2]$,	214 White	52.30(52.51)	3.88(3.97)	5.41(6.12)	7.26(7.75)	22.15(22.63)	890(914.91)

TABLE 2: ^{1}H NMR spectral data (δ, ppm) of the lead (II) compounds

Compound	Phenanthrene moiety	(-CO-NH-)	CO-$(CH_2)_x$-CO-
[Pb(Mac^1)Cl_2],	7.41 - 9.10	7.90	2.80
[Pb(Mac^2)Cl_2],	7.53 - 9.15	7.99	3.09
[Pb(Mac^3)Cl_2],	7.55 - 9.16	8.11	3.11
[Pb(Mac^4)Cl_2],	8.50 - 9.10	8.17	3.20

TABLE 3 : X-Ray diffraction data of the compound [Fe(Mac^2)Cl_2]

Peak no.	2θ obs	2θ calcd	d-spacing obs	h	k	l
1.	17.90	17.86	6.227	5	0	1
2.	17.90	18.04	6.227	0	2	1
3.	19.10	19.01	5.839	5	1	0
4.	19.10	19.29	5.839	2	2	1
5.	21.60	21.50	5.170	3	1	3
6.	22.40	22.51	4.987	0	0	4
7.	26.10	25.97	4.290	7	1	1
8.	26.90	26.73	4.165	7	1	1
9.	28.60	28.43	3.922	0	2	4
10.	32.20	32.22	3.493	8	0	3
11.	34.70	34.53	3.248	0	3	4
12.	34.70	34.76	3.248	10	0	1
13.	34.70	34.70	3.248	1	3	4
14.	42.50	42.34	2.673	1	2	2

Refined Values,a=32.872, b=13.001 and c=19.850, α=β=γ=90° respectively max dev. of 2θ=0.9

lecular weights of the complexes indicate the monomeric nature of the complexes. Elemental analysis agree well with the stoichiometry and chemical formula of the compound [M (Mac^n)Cl_2].

Infrared spectra

The infrared spectra of the starting materials and their metal complexes were studied and some important features may be summerized as follows :

The IR spectra of 9,10-diaminophenanthrene and dicarboxylic acids show the bands due to hydroxyl and amino group, which disappear in corresponding metal complexes, indicating the condensation of amines with the dicarboxylic acids and formation of the proposed macrocyclic frame work. The spectra of all the complexes show a medium intensity band at 3134-3280 cm^{-1} which is assigned to ν(NH) mode of the amide group[30]. The amide I, amide II, amide III and amide IV bands appear at 1647-1788, 1430-1475, 1245-1278 and 639-671 cm^{-1}, respectively[31]. The bands in the region 312-358cm^{-1} in the spectra of all the complexes may be attributed to the M-N stretching vibrations[32]. The M-Cl stretching vibrations have been assigned at 430-461 cm^{-1} as reported by others also[32].

Electronic spectra

The electronic spectra of iron (II) tetraazama crocyclic complexes exhibit a weak intensity band in the region 845-889 nm, which may be assigned to the $5_{T2g} \rightarrow 5_{Eg}$ transitions consistant with an octahedral geometry[33].

^{57}Fe Mossbauer spectra

The mossbauer spectra of the iron (II) complexes have been recorded. The chemical isomer shift values (δ) relative to natural iron foil, which are sensitive to both the oxidation and spin states of iron, are in agreement with the structural assignments made on the basis of above spectral evidences. The value of isomer shift (0.25-0.30 mm s^{-1}) and quadrupole splittings (0.65 mm s^{-1}) at the room temperature are characteristic of six-coordinated low spin iron (II) complexes[34].

^{1}H NMR spectra

The ^{1}H NMR spectra of the lead(II) complexes were recorded in DMSO-d_6 and the chemical shift values δ for the different protons are given in TABLE 2. The following points, which confirm the suggested structures for the lead(II) complexes, are worth mentioning. The ^{1}H NMR spectra of the complexes do not show any signal corresponding to the amino and hydroxy groups. The broad signal observed in all the complexes at δ 7.90-8.17 ppm is due to the amide (CO-NH) protons.

^{13}C NMR spectra

The conclusion drawn from the IR and ^{1}H NMR spectraare parallel with the carbon-13 spectral data regarding the authenticity of the proposed skelton.

X-Ray diffraction spectra

In order to ascertain the lattice dynamics of these compounds X-ray diffraction of the compound [Fe(Mac^2)Cl_2] has been recorded. The observed interplanar spacing values ('d' in Å) have been measured from the diffractogram of the compound and the miller indices h, k and l have been assigned to each d value and 2θ angles are reported in TABLE 3. The results show that the compound, belongs to 'orthorhombic' crystal system having unit cell parameters as

a=32.872, b=13.001 c=19.850. and $\alpha = \beta = \gamma = 90°$, respectively, max. dev. of 2θ=0.9.

Mass spectra

The mass spectrum of the $[Pb(C_{40}H_{36}N_4O_4)Cl_2]$ complex showed the molecular ion peak at m/z 915 $[M]^+$. The one and two coordinated chloride ions are removed with a mass loss of m/z 35 and 70. The molecular cations during fragmentation process loss the coordinated exocyclic ligands under FAB conditions to give species such as : 803 $[Pb(C_{36}H_{28}N_4O_4)Cl_2]^+$; 691 $[Pb(C_{32}H_{20}N_4O_4)Cl_2]^+$; 747 $[Pb(C_{34}H_{28}N_4O_2)Cl_2]^+$ and 707 $[[Pb(C_{26}H_{24}N_2O_4)Cl_2]^+$ from the loss of the C_4H_8, C_8H_{16}, $C_6H_8O_2$ and $C_{14}H_{12}N_2$ fragments, respectively.

^{207}Pb NMR spectra

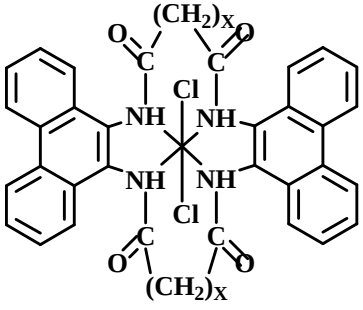
The ^{207}Pb NMR spectra of the complexes give signals at δ2222-2240 ppm, indicating coordination number six in the complexes around lead atom. These results are found tobe in accordance with the results by West et al[35].

The most suitable structures for these derivatives considering their physical measurements, analytical data and spectral evidences are depicted in Figure 1.

Biological aspects

(A) Antifungal activity

Antifungal activity measured is presented in the TABLES 4-8.The results showed that the spore germination inhibited significantly even at the lowest concentration T_4 (50 ppm). Similar results were obtained when 4 selected fungi were taken for their mycelial growth on potato dextrose broth supplemented with the chemicals. The spores which showed sensitivity



where M = Fe(II) and Pb(II) X = 1,2,3 or 4

Figure 1: Proposed structures of the complexes

TABLE 4: Effect of [Fe (Mac^2)Cl_2] on spore germination of some fungi

Fungus/ Treatment	Host	Control	R_1 250 ppm	R_2 125 ppm	R_3 62.5 ppm
Fusarium udum (ATCC 10245)	*Canfanus cajan*	96.73	2.51**	11.25**	18.86**
Alternaria triticina (ATCC 698)	*Triticum aestivum*	99.27	18.03**	24.43**	70.15**
Alternaria brassicae (ATCC 1050)	*B.campestris var. capitata*	99.53	2.00**	4.18**	17.37**
Curvularia lunata (ATCC 10021)	*Oxyza sativa*	97.33	68.89**	82.73	87.54
Curvularia sp. (ATCC 12097), Helminthosporium oryzae (ATCC 11779),	*Brassica campestris*	96.72	2.97**	5.67**	6.59**
Aspergillus flavus (ATCC 17884),	*Oxyza sativa*	96.82	2.99**	5.98**	6.88**
Alternaria brasicicola (ATCC 29739)	*Saprophyte*	80.33	3.17**	8.33**	15.83**
	B.Campestris	92.63	7.56**	11.24**	27.25**

Row data with **are significant at P≥ 0.01

TABLE 5: Effect of the [Fe(Mac^3)Cl_2] on spore germination of some fungi

Fungus/Treatment	Host	Control	S_1 500ppm	S_2 250 ppm	S_3 125 ppm
Fusarium udum (ATCC 10245)	*Canfanus cajan*	98.63	9.78**	15.22**	41.25**
Alternaria triticina (ATCC 698)	*Triticum aestivum*	99.27	49.39**	71.83**	87.87**
Alternaria brassicae (ATCC 1050)	*B.campestris var. capitata*	95.53	14.14**	21.69**	33.49**
Curvularia lunata (ATCC 10021)	*Oxyza sativa*	96.44	73.00**	82.73	87.54
Curvularia sp. (ATCC 12097), Helminthosporium oryzae (ATCC 11779),	*Brassica campestris*	96.33	13.21**	24.86**	36.72**
Aspergillus flavus (ATCC 17884),	*Oxyza sativa*	97.29	6.57**	9.28**	52.15**
Alternaria brasicicola (ATCC 29739)	*Saprophyte*	80.33	7.67**	24.83**	47.00**
	B.Campestris	92.63	24.89**	34.40**	41.87**

Row data with ** are significant at ≥ 0.01.

against the chemicals also showed a similar trend in the production of mycelial dry weight. Out of the 4 tested fungi, Alternaria triticina showed maximum sensitivity when the chemicals were mixed, followed by Alternaria brassicae and Fusarium udum (TABLE 4).

The results of the present experiments showed the probable synergistic effect of the two compounds in the mixture. Such compounds may inhibit development of resistance since they have multisite action majority in comparison to widely used fungicides with single site of action. Further experimentation with these compounds in glasshouse and under field conditions is suggested for practical application of plant disease control.

In case of Fusarium udum and Aspergillus niger the

TABLE 6: Effect of [Fe(Mac^4)Cl$_2$] complex on spore germination of some fungi

Fungus / Treatment	Host	Control	T_1 (400 ppm)	T_2 (200 ppm)	T_3 (100 ppm)	T_4 (50 ppm)
Fusarium udum (ATCC 10245)	*Canfanus cajan*	98.63	4.72**	9.54**	17.34**	24.08**
Alternaria triticina (ATCC 698)	*Triticum aestivum*	93.32	0.60**	10.72**	27.31**	63.11**
Alternaria brassicae (ATCC 1050)	*B.campestris var. capitate*	92.47	2.18**	4.92**	12.53**	20.16**
Curvularia lunata (ATCC 10021)	*Oxyza sativa*	91.39	3.94**	11.19**	23.32**	37.23**
Curvularia sp. (ATCC 12097),	*Brassica campestris*	86.39	1.18**	3.37**	10.74**	20.59**
Helminthosporium oryzae (ATCC 11779),	*Oxyza sativa*	84.78	3.44	4.93**	8.58**	17.97**
Aspergillus flavus (ATCC 17884),	*Saprophyte*	94.32	6.17**	13.86**	26.06**	29.20**
Alternaria brasicicola (ATCC 29739)	*B.Campestris*	92.63	11.52**	21.58**	29.95**	37.76**

Row data with ** are significant at p ≥ 0.01

TABLE 7 : Effect of the [Fe (Mac^2)Cl$_2$], [Fe(Mac^3)Cl$_2$] and [Fe(Mac^4)Cl$_2$] complexes on mycelial growth of some fungi

Treatment	Conc. (ppm)	*Curvularia lunata (ATCC 10021)*	*Fusarium udum (ATCC 10245)*	*Alternaria brassicae (ATCC 1050)*	*Alternaria triticina (ATCC 698)*
Control		0.2301	0.2195	0.2170	0.2486
	100	0.1889	0.1487**	0.1299**	0.1440**
[Fe(Mac^2)Cl$_2$]	200	0.1320**	0.1138**	0.1140**	0.1145**
	400	0.1024**	0.1015**	0.0982**	0.1021**
	100	0.1772**	0.1576**	0.2277**	0.2135**
[Fe(Mac^3)Cl$_2$]	200	0.1488**	0.1266**	0.2212**	0.1875**
	400	0.0996**	0.1075**	0.2100**	0.1477**
	100	0.1455**	0.1185**	0.1010**	0.1245**
[Fe(Mac^4)Cl$_2$]	200	0.1241**	0.0983**	0.8260**	0.0930**
	400	0.0857**	0.0481**	0.0496**	0.0230**

Column data with ** are significant at P ≥ 0.01.

effect of the [Fe (Mac^3)Cl$_2$],[Fe (Mac^2)Cl$_2$] and [Fe (Mac^4)Cl$_2$] were very significant showing inhibition upto 100% in many these cases (TABLE 5).

From an overall study of the effect of the [Fe (Mac^2)Cl$_2$], [Fe (Mac^3)Cl$_2$] and [Fe (Mac^4)Cl$_2$] inferred that all the complexes are good fungicidal agent. Again in certain cases the complex, [Fe (Mac^2)Cl$_2$] is more effective i.e., show more fungi-toxicity in comparison to the individual [Fe (Mac^2)Cl$_2$] or the [Fe (Mac^3)Cl$_2$. For practical utility of this compound, the inhibiting capacity of the complex was compared with the commercially available fungicide, dithane – M-45 (a broad fungicide) which is used in the inhibition of spore germination in the 0.1-0.2% in the field condition limit for many fungi. It was found that in the case of Ph_3SnL_8 against Fusarium udum and Curvularia species, the effect of the complex was found to be better than that of

TABLE 9 : Antibacterial screening data of the iron(II) and Pb(II) complexes. Diameter of inhibition zone (mm

Compound	*P.cepacicola (ATCC 1079)*		*E.coli (ATCC 1920)*		*S.aureus (ATCC 1450)*		*K.aerogenous (ATCC 793)*	
	500	**1000**	**500**	**1000**	**500**	**1000**	**500**	**1000**
[Fe(Mac^1)Cl$_2$],	9	10	11	12	11	12	12	13
[Fe(Mac^2)Cl$_2$],	5	7	3	7	4	6	3	7
[Fe(Mac^3)Cl$_2$],	10	13	13	14	12	14	13	14
[Fe(Mac^4)Cl$_2$],	11	12	11	13	11	13	10	11
[Pb(Mac^1)Cl$_2$],	11	13	13	13	12	14	13	15
[Pb(Mac^2)Cl$_2$],	9	12	8	10	9	14	5	8
[Pb(Mac^3)Cl$_2$],	12	13	12	14	12	14	11	12
[Pb(Mac^4)Cl$_2$],	13	14	14	15	13	14	12	14
Standard (Streptomycin)	15	16	17	18	15	17	13	15

TABLE 8 : Effect of the [Fe (Mac^2)Cl$_2$], [Fe (Mac^3)Cl$_2$] and [Fe (Mac^4)Cl$_2$] complexes on spore germination of some fungi (% inhibition)

Fungus/Treatment	Host	Control	[Fe (Mac^2)Cl$_2$]		[Fe (Mac^3)Cl$_2$]		[Fe (Mac^4)Cl$_2$]	
			1000	**500**	**1000**	**500**	**1000**	**500**
Fusarium udum (ATCC 10245)	*Canfanus cajan*	1.35	98.65	90.0	99.37	88.2	100.0	95.4
Alternaria brassicae (ATCC 1050)	*B.campestris var. capitata*	7.35	26.0	4.5	15.0	3.4	86.6	78.6
Curvularia lunata (ATCC 10021)	*Oxyza sativa*	32.20	99.05	92.6	96.2	82.5	100.0	96.2
Curvularia sp. (ATCC 12097),	*Brassica campestris*	3.65	98.77	85.19	99.4	85.3	100.0	98.8
Helminthosporium oryzae (ATCC 11779),	*Oxyza sativa*	1.18	100.0	98.4	26.2	4.3	91.3	77.6
Aspergillus flavus (ATCC 17884),	*Saprophyte*	19.80	99.7	92.27	56.7	27.4	99.2	94.8
Alternaria brasicicola (ATCC 29739)	*B Campestris*	22.24	99.5	92.5	48.2	22.4	77.2	54.2

TABLE 10 : Body and organs weight

Treatment	Body weight(gm)		Tissue Weight (mg/100 gm body weight)							
	Initial	Final	Testes	Epididymis	Seminal vesicle	Ventral prostate	Vas deferencs	Kidney	Adrenal	Liver
Group - I 0.5 ml olive oil/day	192.50± 5.45	212.50 ± 4.51	1032.09 ± 12.50	298.13 ± 4.86	237.21 ± 8.33	160.48 ± 4.32	72.05 ± 1.50	490.06 ± 14.36	29.41± 0.44	3734.64 ± 181.23
Group - II 5 mg/kg b.w.t./day	186.70± 18.00	206.00 ± 17.04	641.09* ± 26.87	243.13*± 5.78	207.21* ± 14.28	128.92* ± 1.34	40.04** ± 0.89	378.28* ± 14.13	27.23^{ns} ± 0.62	3711.11^{ns} ± 105.45
Group - III 5 mg/kg b.w.t./day	186.25± 3.35	208.00 ± 8.62	545.16** ± 12.19	208.21**± 9.20	158.97** ± 8.00	127.27** ± 5.05	37.30** ± 0.32	333.26** ± 5.20	23.36* ± 0.94	3310.54* ± 336.73
Group - IV 5 mg/kg b.w.t./day	190.50± 8.84	212.00 ± 9.87	489.03** ± 12.50	191.95**± 4.86	138.43** ± 8.33	95.41** ± 3.82	42.05** ± 1.50	331.22** ± 4.36	20.07** ± 0.44	2871.11** ± 181.90
Group - V 5 mg/kg b.w.t./day	185.00± 6.96	202.75 ± 8.70	549.35** ± 8.62	203.39 ± 2.88	149.67** ± 4.27	109.49** ± 0.93	33.41** ± 2.73	354.78* ± 16.22	21.23** ± 0.69	3139.64**± 61.09
Group - VI 5 mg/kg b.w.t./day	187.50± 5.38	205.00 ± 17.38	451.08** ± 6.23	162.75** ± 1.86	123.73** ± 4.82	97.45** ± 3.29	41.24** ± 0.80	287.99** ± 13.43	20.58** ± 0.89	2788.60** ± 118.17

(mean ± SEM of 6 Animals), ns=Non-significant, *=P ≤ 0.01=Significant, **=P ≤ 0.001=Highly-Significant, Group-I=Control, Group-II== [Fe (Mac^2)Cl$_2$], Group-III== [Fe (Mac^3)Cl$_2$], Group-IV== [Fe (Mac^4)Cl$_2$], Group-V= [Pb (Mac^2)Cl$_2$], Group-VI= [Pb (Mac^4)Cl$_2$]

TABLE 11 : Sperm dynamics and fertility test

Treatment	No. of males	No. of females	No. of pregnant females	Sperm motility (Cauda epididymis) (%)	Sperm density (Cauda epididymis) (million/ml)	Fertility test (%)
Group - I 0.5 ml olive oil/day	6	12	12/12	66.73±1.33	54.50±0.49	100 (+ve)
Group - II 5 mg/kg b.w.t./day	6	12	8/12	48.59±1.67	31.69**± 2.48	35 (-ve)
Group - III 5 mg/kg b.w.t./day	6	12	5/12	38.84±2.36	18.17 ± 3.39	60 (-ve)
Group - IV 5 mg/kg b.w.t./day	6	12	3/12	35.45**± 2.35	14.50** ± 0.29	75 (-ve)
Group - V 5 mg/kg b.w.t./day	6	12	4/12	30.65±3.66	17.22 ** ± 0.69	70 (-ve)
Group - VI 5 mg/kg b.w.t./day	6	12	1/12	26.59** ± 1.67	10.01** ± 0.22	90 (-ve)

(Mean ± SEM of 6 Animals, **=P ≤ 0.01=Hihgly-significant., All Groups Compared with the control, Group-I=Control , Group-II= [Fe (Mac^2)Cl$_2$], Group-III= [Fe (Mac^3)Cl$_2$], Group-IV= [Fe (Mac^4)Cl$_2$], Group-V= [Pb (Mac^2)Cl$_2$], Group-VI= [Pb (Mac^4)Cl$_2$]

commercially available fungicide dithane M-45.

This observation is quite significant and opens, up a new field of research as the metal complex [Fe(Mac^4)Cl$_2$] is better fungi toxic than commercial products, showing greater possibility of applicability of the complex under field conditions.

(B) Antibacterial activity

The antibacterial activity of the few representative ligands and their metal/metalloid complexes has been screened against various bacteria and the results are recorded in TABLE 9. The experimental results show that the metal chelates are more potent in inhibiting the growth of microorganism than the original ligands. The enhanced antimicrobial activity of the metal chelates over their corresponding chelating agents may be explained by the chelation theory. Chelation reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups and possible π-electron delocalization over the whole chelate ring. This increase the lipophilic character of the metal complexes, which subsequently favour its permeation through the semipermeable defenses of cell membrane of microorganism and thereby, impairing the normal cell process.

(C) Antifertility activity

The results reported in TABLES 10 and 11 revealed that there is a highly significant reduction (P<0.001) in the sperm motility. A severe impairment of sperm density in cauda epididymus was observed after 60 days

of treatment as compared to the control group. As the cauda epididymal sperm suspension of control rat revealed that the sperms were actively motile, showing forward progression .The sperm motility of compound treated rats showed that the sperms were sluggishly motile without forward progression.

Body and organ weight

The oral treatment of complexes did not cause any significant change in the body weight of the treated rats with respect to initial body weight. However the weight of testes, epididymus, seminal vesicle, ventral prostrate and vasa deferens decreased highly significantly ($P<0.001$) with respect to the weights of control group animals.

Sperm motility and sperm density

The results reported in TABLE 11 reveal that there is a highly significant reduction ($P<0.001$) in the sperm motility. A severe impairment of sperm density in cauda epididymus was observed after the 60 days of treatment as compared to the control group. As the caudaepididymal sperm suspension of control rat revealed that the sperms were actively motile, showing forward progression. The sperm motility of compound treated rats showed that the sperms were sluggishly motile without forward progression.

Biochemical parameters[36]

The oral administration of complexes in male albino rats caused a highly significant ($P<0.001$) increase in the testes cholesterol levels in the rats. The liver cholesterol level was unaffected in all the treated groups. A highly significant decrease ($P<0.001$) was observed in the testes glycogen level of the animals treated with the complexes.

A non- significant change in the liver glycogen was observed in all the treated groups

Hematology

The values of RBC and WBC counts, Hemoglobin and hematocrit[31] values remained unaltered indicating normal blood physiology. The values of M.C.H, M.C.V and M.C.H.C were also unaffected following the treatment.

CONCLUSION

The present results demonstrate the effects of these compounds on the male reproductive system of rats and other physiological parameters. Further, the current study strongly demonstrate that the $[Pb(Mac^4)Cl_2]$ complex is more effective antimicrobial and antifertility agent than the other compounds.

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