Scientific paper

Metal Ion Prompted Macrocyclic Complexes Derived from Indole-2,3-dione (isatin) and O-phenylenediamine With their Spectroscopic and Antibacterial Studies

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Abstract

A novel series of the complexes of the type:$[M(TML)X]X_2$; where TML is a tetradentate macrocyclic ligand; M=Cr(III), Fe(III); X=Cl^-, NO_3^-, OAc^- has been synthesized by condensation of indole-2,3-dione (isatin) and o-phenylenediamine in the methanolic medium. The complexes have been characterized with the help of various physicochemical techniques like elemental analyses, conductance measurements, magnetic measurements, infrared and electronic spectral studies. Molar conductance values indicate they are 1:2 electrolytes. Electronic spectra along with magnetic moments suggest the five coordinate square-pyramidal geometry for these complexes. The complexes were also tested for their *in vitro* antibacterial activities. Some of the complexes showed remarkable antibacterial activities against some of the selected bacterial strains.

Keywords: Macrocyclic complexes, Cr(III) and Fe(III), template synthesis, antibacterial.

1. Introduction

The design and study of well-arranged metal-containing macrocycles is an interesting field of chemistry.[1] Several synthetic and natural macrocyclic compounds have been investigated.[2] The chemistry of macrocyclic complexes has attracted the interest of both inorganic and bioinorganic chemists in recent years because of its importance in the area of coordination chemistry.[3,4] Macrocyclic compounds and their derivatives are interesting ligand-system because they are good hosts for metal anions, neutral molecules and organic cation guests and are very useful in fundamental studies e.g. in phase transfer catalysis and biological studies.[5,6] The family of complexes with aza-macrocyclic ligands has remained a focus of scientific attention for many decades.[7] *In-situ* one pot template condensation reactions have been widely used for the synthesis of macrocyclic complexes, where generally the transition metal ions are used as templating agent.[8,9,10] The metal ions direct the reaction preferentially towards cyclic rather than oligomeric or polymeric product.[11] Synthetic macrocyclic complexes mimic some naturally occurring macrocycles because of their resemblance with many natural macrocycles like metalloproteins, porphyrins and cobalamine.[12–13] Transition metal macrocyclic complexes have received a great attention because of their biological activities, including antiviral, anticarcinogenic,[13] antifertile,[14] antibacterial and antifungal.[15] Some lanthanides macrocycles e.g. Gd^{3+} are used as MRI contrast agents.[16] Macrocyclic metal chelating agents are useful for detecting tumour lesions.[17] The macrocyclic complexes are also used as NMR shift reagent.[18] Some macrocyclic complexes have received special attention because of their mixed soft-hard donor character and versatile coordination behavior.[19] Cu(II) complexes with isatin Schiff base ligands are potential antitumoral agents.[20] In the previous work, we have reported the macrocyclic complexes derived from indole-2,3-dione (isatin) and ethylenediamine with divalent metal salts.[21] On the basis of above studies, in the present paper, macrocyclic complexes of Cr(III) and Fe(III) derived from indole-2,3-dione (isatin) and o-phenylenediamine are prepared, characterized by standard physicochemical tech-

nique, tested for their *in vitro* antibacterial activities and discussed.

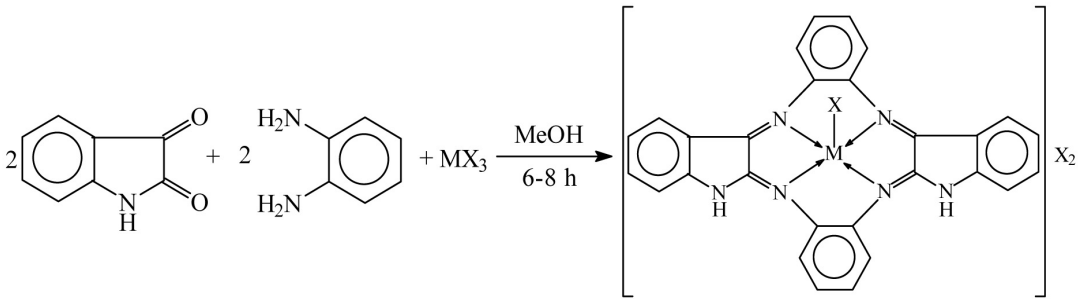
2. Experimental

All the chemicals used were of Anal R grade. Metal salts were purchased from E. Merck and were used as received.

2. 1. Isolation of Complexes

Various attempts to isolate the free macrocyclic ligand were unsuccessful. Hence, all the complexes were synthesized by template synthesis. To a hot, well stirred methanol solution (~50cm^3) of o-phenylenediamine (10 mmol), trivalent chromium or iron salts (Cl^-, NO_3^-, OAc^-) (5 mmol) dissolved in minimum quantity of methanol were added. The resulting solution was refluxed for 0.5 h. After that, indole-2,3-dione (isatin) (10 mmol) dissolved in ~20 cm^3 methanol was added in the reaction mixture and refluxing continued for 6–8 h. The mixture was concentrated to half of its volume and kept in desiccator for 2 days. The complexes were then filtered, washed with methanol, acetone and diethylether and dried *in vacuo*. The complexes were found soluble in DMF and DMSO, but were insoluble in water. They were found thermally stable up to ~240 °C and then decomposed.

The syntheses of the complexes may be represented by the following scheme:



Where M = Cr(III) and Fe(III) and X = Cl^-, NO_3^-, OAc^-

2. 2. Analytical and Physical Measurements

The microanalyses of C, H, and N were carried out at Sophisticated Analytical Instrument Facility, CDRI, Lucknow. Melting points were determined using capillaries in electrical melting point apparatus. The metal contents were determined by standard EDTA methods. The magnetic susceptibility measurements were carried out at SAIF, IIT Roorkee on vibrating sample magnetometer. Electronic spectra (DMF) were recorded on Cary 14 spectrophotometer. The IR spectra were recorded on Infrared Spectrophotometer in the range 4000–200 cm^{-1} using Nujol Mull/KBr pellets. The conductivity was measured at room temperature in DMSO on digital conductivity meter (HPG System, G-3001).

3. Biological Assay

The synthesized macrocyclic complexes were tested for *in vitro* antibacterial activity against some bacterial strains using spot-on-lawn on Muller Hinton Agar.[22]

3. 1. Test Pathogens

Five test pathogenic bacterial strains *viz. Staphylococcus aureus, Bacillus pumilus, Bacillus megaterium*, and *Staphylococcus epidermidis* (Gram-positive) *Pseudomonas aeruginosa* (Gram-negative) were considered for determination of MIC (Minimum Inhibitory Concentration) of selected complexes.

3. 2. *In-vitro* Antibacterial Activity

3. 2. 1. Culture Conditions

The test pathogens were subcultured aerobically using Brain Heart Infusion Agar (HiMedia, Mumbai, India) at 37 °C for 24 h. Working cultures were stored at 4 °C in Brain Heart Infusion (BHI) broth (HiMedia, Mumbai, India), while stock cultures were maintained at –70 °C in BHI broth containing 15% (v/v) glycerol (Qualigens, Mumbai, India). Microorganism was grown overnight in 10 ml BHI broth, centrifuged at 5,000g for 10 min and the pellet was suspended in 10 ml of phosphate buffer saline (PBS, pH 7.2). Optical density at 545 nm (OD-545) was adjusted to obtain 108 cfu/ml followed by plating serial dilution onto plate count agar (HiMedia, Mumbai, India).

3. 2. 2. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration is the lowest concentration of the antimicrobial agent that prevents the visible growth of the microorganisms after overnight incubation. MIC of synthesized complexes against above

mentioned bacteria were determined. Muller Hinton Agar (MHA) was used for MIC determination.[22] All the test cultures were streaked on the Soyabean Casein Digest Agar (SCDA) and incubated overnight at 37 °C. Turbidity of all the bacterial cultures was adjusted to 0.5 McFarland by preparing bacterial suspension of 4–6 well isolated colonies of the same morphological type selected from a SCDA plate. The cultures were further diluted 10 folds to attain the inoculum size of 1.4×10^7 cfu/mL. Stock solutions of 4 mg/mL of all complexes were prepared in DMSO and were appropriately diluted to get final concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.12 mg/mL. Standard antibiotics, *viz. Linezolid* and *Cefaclor* were also diluted in the same manner for comparison. A 20 mL of each dilution was added to 20 mL of cooled to 45 °C molten MHA (separate flask was taken for each dilution). After thorough mixing, the medium was poured in sterilized Petri plates. The test bacterial cultures were spotted in a pre-defined pattern by aseptically transferring 10 mL of each culture onto the surface of pre-solidified agar plates. The spotted plates were incubated at 35 °C for 24 h. The concentration at which growth of the bacterial cultures was inhibited completely gives MIC of test complexes. Zone of inhibition of complexes were considered after subtraction of inhibition zone of DMSO. Negative control (with no complex) was also observed. All the experiments were performed in triplicates.

4. Results and Discussion

The analytical data show the formula for macrocyclic complexes as: $[M(C_{28}H_{18}N_6)X]X_2$; where M=Cr(III) and Fe(III) and X=Cl^-, NO_3^-, OAc^-. Conductivity measurement in DMSO (Table-1) indicated that they are 1:2 electrolytes (150–180 S cm^2 mol^{-1}).[23] Various attempts such as crystallization using mixtures of solvents, low temperature crystallization were unsuccessful to obtain a single crystal suitable for X-ray crystallography. However, the analytical, spectroscopic and magnetic data enable us to predict the possible structure of the synthesized complexes. All complexes give satisfactory elemental analyses results as shown in Table -1.

4. 1. *IR* Spectra

The presence of single medium intensity bands in the region ~3243–3300 cm^{-1} in the complexes may be assigned due to N–H valence stretching vibration. It was noted that a pair of medium intensity bands corresponding to $\nu(NH_2)$ are present at 3250 and 3280 cm^{-1} in the spectrum of o-phenylenediamine but were absent in the infrared spectra of all the synthesized complexes due to condensation of amino group with keto group. A strong peak was observed at ~1732 cm^{-1} in the spectrum of indole-2,3-dione (isatin) which may be attributed due to >C=O group. This peak was absent in the spectra of all the metal complexes. This indicate the absence of >C=O group of indole-2,3-dione (isatin) moiety in all the complexes and indicates the condensation of carbonyl group of indole-2,3-dione (isatin) and amino group of o-phenylenediamine.[24,25] A strong absorption band was appeared in the region ~1590–1615 cm^{-1} which may be assign to ν(C=N).[26,27] These results provide strong evidence for the formation of macrocyclic frame.[28,29] The lower values of ν(C=N) may be explained on the basis of drift of lone pair density of azomethine nitrogen towards metal atom.[30,31] Another set of medium intensity bands in the region ~1500–1590 cm^{-1} are attributed to ν(C=C) of phenyl groups and bands at ~840–880 cm^{-1} to (C–H) out of plane bending of phenyl groups. The valence stretching vibrations of (C–N) occur in the range ~1360–1015 cm^{-1}.

The far infrared spectra show bands in the region ~425–460 cm^{-1} corresponding to ν(M–N) stretching vibrations in all the complexes.[32] The presence of these bands in all complexes suggesting coordination of azomethine nitrogen.[33] The bands present at 295–315 cm^{-1} correspond to ν(M–Cl) stretching vibrations.[32,33] The bands

Table 1. Analytical data of trivalent chromium and iron complexes derived from indole-2,3-dione (isatin) and o-phenylenediamine.

Sr. No.	Complexes	Found (Calcd.) %				Colour	Mol. Wt.	Yield (%)	Molar Conductance (S cm^2 mol^{-1})
		M	C	H	N				
(1)	$[Cr(C_{28}H_{18}N_6)Cl]Cl_2$	8.33 (8.72)	55.93 (56.33)	2.72 (3.01)	13.69 (14.08)	Brown	596.5	42.99	175
(2)	$[Cr(C_{28}H_{18}N_6)(NO_3)](NO_3)_2$	7.21 (7.69)	49.47 (49.70)	2.19 (2.66)	18.44 (18.64)	Dark brown	676	43.25	169
(3)	$[Cr(C_{28}H_{18}N_6)(OAc)](OAc)_2$	7.58 (7.80)	60.78 (61.17)	3.59 (4.05)	12.19 (12.59)	Yellowish brown	667	39.37	150
(4)	$[Fe(C_{28}H_{18}N_6)Cl]Cl_2$	9.24 (9.33)	55.79 (55.95)	2.91 (2.99)	13.88 (13.99)	Reddish brown	600.5	42.17	180
(5)	$[Fe(C_{28}H_{18}N_6)(NO_3)](NO_3)_2$	7.92 (8.24)	48.83 (49.41)	2.15 (2.65)	18.02 (18.53)	Dark brown	680	43.18	165
(6)	$[Fe(C_{28}H_{18}N_{6)}(OAc)](OAc)_2$	7.87 (8.35)	60.14 (60.80)	3.86 (4.02)	12.11 (12.52)	Orange red	671	40.00	160

present at 210–250 cm^{-1} in all nitrate complexes are assigned due to ν(M–O)[33] stretching vibrations.

4. 2. Magnetic Measurements and Electronic Spectra

4. 2. 1. Chromium Complexes

The magnetic moments of chromium complexes at room temperature was found in the range of 3.85–4.22 μ_B, which was close to the predicted values for three unpaired electrons in the Cr(III) metal ion.[34] The electronic spectra of chromium(III) complexes showed bands at ~9080–9330cm^{-1} (ν_1), ~13100–13400 cm^{-1}(ν_2) , ~17350–18200 cm^{-1}(ν_3), ~27200–27600 cm^{-1}(ν_4) and ~34700 cm^{-1}(ν_5), respectively. These spectral bands were comparable with that of five coordinated Cr(III) complexes whose structure has been confirmed with the help of X-ray measurements.[35] On the basis of the analytical data, spectral studies and electrolytic nature of these complexes, a five coordinated square-pyramidal geometry may be assigned for these complexes.[34,36] Thus, asuming the symmetry C_{4V} for these complexes, various spectral bands may be assigned as: $^4B_1 \rightarrow {}^4E^a$, ($\nu_1$); $^4B_1 \rightarrow {}^4B_2$, ($\nu_2$); $^4B_1 \rightarrow {}^4A_2$, ($\nu_3$) and $^4B_1 \rightarrow {}^4E^b$ (ν_4), respectively.

4. 2. 2. Iron Complexes

The magnetic moments of iron complexes was found in the range of 5.75–5.92 μ_B, corresponding to the five unpaired electrons and was close to the predicted high spin values for these metal ions.[34] The electronic spectra of trivalent iron complexes showed various bands at ~ 9850–9950 cm^{-1}, 15,400–15,800 cm^{-1} and 27,500–27,800 cm^{-1}. The spectral bands were consistent with the range of spectral bands reported for five coordinated square-pyramidal iron(III) complexes.[34,36] Assuming C_{4V} symmetry for these complexes, various bands may be assigned as: $d_{xy} \rightarrow d_{yz,\ xz}$ and $d_{xy} \rightarrow d_{z^2}$. Any attempt to make accurate assignment was difficult due to interactions of the metal-ligand-π bond systems lifting the degeneracy of $d_{xz \text{ and }} d_{yz}$ pair.

4. 3. Biological Results and Discussion

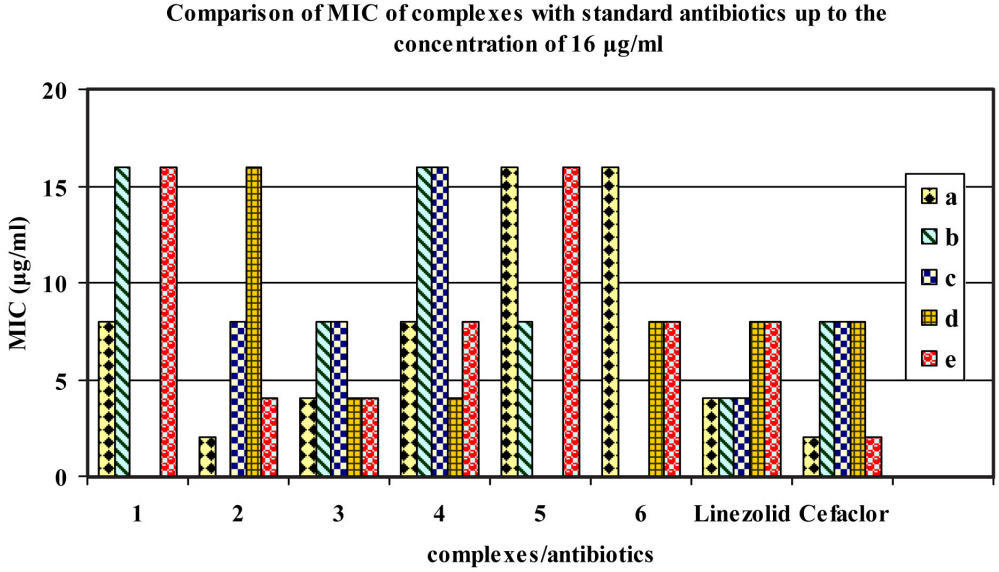
All the chemically synthesized complexes were tested *in vitro* for their antibacterial activity as shown in Table-2. All the complexes of the tested series possessed high antibacterial activities against all the bacterial strains. The MIC (minimum inhibitory concentration) shown by the complexes was compared with MIC obtained by standard antibiotics *Linezolid* and *Cefaclor* (Table-2, Fig.-1). Among the series under test for determination of minimum inhibitory concentration, complexes (2), (3) and (6) was found to be most potent complexes as they showed MIC equal to that of standard antibiotic

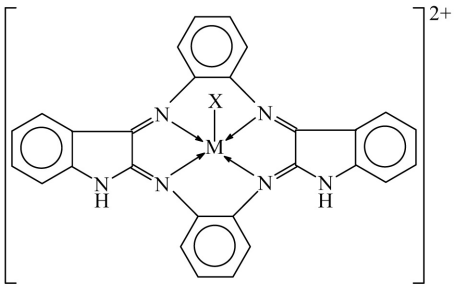
Table 2. Minimum inhibitory concentration (MIC) (in μg/ml) of the complexes by using macro dilution method.

Sr. No.	Complexes	MIC (μg/ml) a	b	c	d	e
(1)	$[Cr(C_{28}H_{18}N_6)Cl]Cl_2$	08	16	32	32	16
(2)	$[Cr(C_{28}H_{18}N_6)(NO_3)](NO_3)_2$	02	32	08	16	04
(3)	$[Cr(C_{28}H_{18}N_6)(OAc)](OAc)_2$	04	08	08	04	04
(4)	$[Fe(C_{28}H_{18}N_6)Cl]Cl_2$	08	16	16	04	08
(5)	$[Fe(C_{28}H_{18}N_6)(NO_3)](NO_3)_2$	16	08	32	32	16
(6)	$[Fe(C_{28}H_{18}N_6)(OAc)](OAc)_2$	16	32	32	08	08
(7)	*Linezolid**	04	04	04	08	08
(8)	*Cefaclor**	02	08	08	08	02

a – *Staphylococcus aureus;* b – *Bacillus pumilus;* c – *Bacillus megaterium*; d – *Pseudomonas aeruginosa;* e – *Staphylococcus epidermidis*

* – Standard antibiotics





Where M = Cr(III) and Fe(III) and X = Cl^-, NO_3^-, OAc^-

Cefaclor and *Linezolid* against different bacterial strains. However, complex (1) and (5) showed poor antibacterial activities against all bacterial strains among the whole series.

5. Conclusion

Therefore based on various physico-chemical techniques like elemental analyses, conductivity measurements, magnetic moments, electronic and infrared spectral studies, a five coordinate square pyramidal geometry for these complex cations may be proposed as shown in Fig.-2.

Among the series under test for determination of minimum inhibitory concentration, all the complexes were not found antibacterial active, however some complexes was found to showed high antibacterial activities as they showed MIC equal to that of standard antibiotic *Cefaclor* and *Linezolid* against different bacterial strains. It has been suggested that chelation/coordination reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with donor group within the whole chelate ring system.[37] This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favours its permeation through the lipoid layer of the membrane thus causing the metal complex to cross the bacterial membrane more effectively thus increasing the activity of the complexes. Besides, many other factors such as solubility, dipole moment, conductivity influenced by metal ion may be the possible reasons for antibacterial activities of these metal complexes.[38] It has been also observed that some moieties such as azomethine linkage or heteroaromatic nucleus introduced into such compounds exhibit extensive biological activities that may be responsible for the increase in hydrophobic character and liposolubility of the molecules in crossing the cell membrane of the microorganism and enhance biological utilization ratio and activity of complexes.[39]

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Abbreviations

CFU-Colony Forming Unit, DMF-N, N dimethylformamide, DMSO-Dimethylsulphoxide, IR-Infrared, MIC-Minimum Inhibitory Concentration, NMR-Nuclear Magnetic Resonance, MHA-Mueller Hinton Agar, MRI-Magnetic Resonance Imaging. BHI-Brain Heart Infusion.

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Povzetek

S kondenzacijo izatina in o-fenilendiamina v metanolu smo pripravili vrsto novih kompleksov tipa $[M(TML)X]X_2$; kjer TML pomeni tetradentatni makrociklični ligand in M=Cr(III), Fe(III); X=Cl^-, NO_3^-, CH_3COO^-. Dobljene komplekse smo preiskovali z uporabo različnih fizikalnokemijskih tehnik (elementna analiza, meritve prevodnosti, NMR, IR in elektronska spektroskopija). Odvisnost molske prevodnosti od koncentracije kaže na to, da komplekse lahko obravnavamo kot 1,2 elektrolite. Elektronska in NMR spektroskopija domnevata petkoordinatno strukturo kvadratne piramide. Komplekse smo tudi *in vitro* testirali za antibakterijsko aktivnost.