Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(1):146-151
(http://derpharmachemica.com/archive.html)

Antimicrobial studies of tetraazamacrocyclic complexes of Fe(III) and Co(II)

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ABSTRACT

*In this communication, electrochemical and antimicrobial studies of tetraazamacrocyclic complexes [$FeC_{42}H_{32}N_4Cl_2$] **[1a]** and [$CoC_{42}H_{32}N_4Cl_2$] **[1b]** have been carried out. The spectral studies were conformed the octahedral geometry of these macrocyclic complexes. Cyclic voltammetric studies were carried out on Pt dish (0.031 cm^2) electrode in DMF. These studies have shown one electron quasirreversible redox processes for these macrocyclic complexes on the basis of their peak separation (ΔE) and peak current ratio (i_{pa}/i_{pc}). Diffusion coefficient (D_0) and heterogeneous electron transfer rate constant (K^0) were also calculated with the help of Nicholson and Kochi's method and found in the order $K^0_{[1b]} > K^0_{[1a]}$. Antimicrobial activity of these macrocyclic complexes was also tested against E. coli, P. aeruginosa, B. cereus, S. aureus and antifungal activity against C. albicans. The observed antimicrobial activity was compared with a standard drug, Gentamycin.*

Keywords: Macrocyclic complexes, spectral studies, cyclic voltammetry, antibacterial activity.

INTRODUCTION

The ability of synthetic macrocyclic complexes to mimic the properties of naturally occurring macrocyclic systems is now well recognized. The design and synthesis mimics of natural macrocyclic systems and to predict their properties [1-5]. There has been much interest in the field of mixed-valence macrocyclic complexes of metal ions. The significant roles of macrocyclic complexes played in living systems by metal ions that depend on their confinement within approximately planar tetradentate totally enclosed highly conjugated macrocyclic framework. Much of the mystique associated with the binding of molecular oxygen by naturally occurring heme, has been removed by successful challenge to this field [6-9]. The biological activity of natural products with medicinally useful properties and some of their derivatives and synthetic analogues furnished the early hypothesis about the possible relationship of chemical structure and overall biological behavior of these substances [10-12].

Tetraazamacrocyclic complexes have been widely studied for their antimicrobial and anticancer properties. Macrocyclic ligands are prominent in naturally occurring ligands in biology, such as the 16-membered inner ring in the porphyrin macrocyclic framework of heme proteins and the 15-membered ring in the Corrine macrocyclic framework of vitamin B_{12} [13]. Currently available, the most potent and specific CXCR4 antagonists are the non peptide bicyclam derivatives macrocyclic complex which were originally developed as antiviral agents blocking the cell entry of T cell tropic HIV strains. The prototype bicyclam macrocyclic complex, AMD3100, is a highly specific CXCR4 antagonist that inhibits binding and function of the natural chemokine ligand system SDF -1a (stromal cell derived factor - 1a) with high affinity and potency. Macrocyclic complexes of Nickel are known to be carcinogenic in humans and experimental animals. The mechanisms leading to tumor formation are not clear, it is confirmed that macrocyclic complexes of nickel can enhance the cytotoxicity and genotoxicity and accumulate in genetic material [14-15].

In this communication, synthesis, characterization, electrochemistry and antimicrobial activity of Dimethyl-dibenzo[b,h]tetraphenyl-2,3,8,9-tetraazacyclododeca-1,3,7,9-tetraene macrocyclic complexes of Fe(III) [1a] and Co(II) [1b] complexes have been carried out.

MATERIALS AND METHODS

The elemental (C, H, N) and mass spectral studies of these macrocycles were carried out at Central Instrumental Laboratory (CIL) Panjab University, Chandigarh (Eager Xperience and TOF MS ES+6018e3). The molar conductance of these macrocycles complexes was recorded on Auto ranging Conductivity/TDS Meter (TCM 15+). The electronic and IR spectra of these macrocyclic complexes were recorded on Double Beam Spectrophotometer (Shimadzu 2450 spectrophotometer) in methanol and Schimadzu-8400S double beam spectrophotometer by KBr DRS method respectively. The electrochemical studies of these macrocyclic complexes were carried on the Platinum electrode (0.031 cm^2) using Auto lab Metrohm 663 VA Stand Instrument. Tetraethylammoniumperchlorate (TEAP) used as supporting electrolytes. The pre-treatment of Pt electrode was carried out before every experiment.

2.1. Preparation of the Macrocyclic complexes:

The macrocyclic complexes have been synthesized according to the literature method [16].

2.1.1. Synthesis of [1a] macrocyclic complex

3,4-diaminotoluene (0.244 g, 2 mole), benzil (0.420 g, 2 mole) and iron (III) chloride (0.162 g, 1 mole) were dissolved in 50 mL methanol in round bottom flask. The reaction mixture was refluxed for 6-8 hours. A dark brown colored reaction mixture was obtained, which then concentrated and the concentrate was kept in desiccator overnight. Dark brown colored crystals of macrocyclic complex appeared, which was then filtered, and washed with methanol, acetone and diethyl ether and dried in vacuum. The crystals were recrystallized, and characterized by spectroscopic techniques like CHN, UV-Vis, FT-IR, and mass spectra. Analytical data is given in **table 1** and scheme for the synthesis of [1a] macrocyclic complex is shown in **fig. 1**.

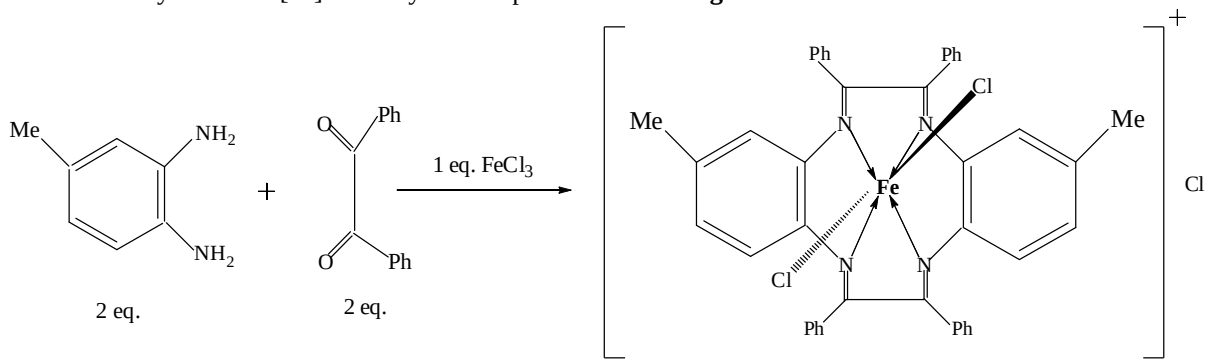


Fig. 1: The synthesis scheme of Dimethyl-dibenzo[b,h]tetraphenyl-2, 3, 8, 9-tetraazacyclododeca-1,3,7,9-tetraene macrocyclic complexes of Fe(III) ($[FeC_{42}H_{32}N_4Cl_2]Cl$) [1a]

2.1.2. Synthesis of [1b] macrocyclic complex

3,4-diaminotoluene (0.244 g, 2 mole), benzil (0.420 g, 2 mole) and cobalt (II) chloride hexahydrate (0.237 g, 1 mole) were dissolved in 50 mL methanol in round bottom flask. Rest of the method was similar as described above. Analytical data is given in **table 1** and scheme for the synthesis of **[1b]** is shown in **fig. 2.**

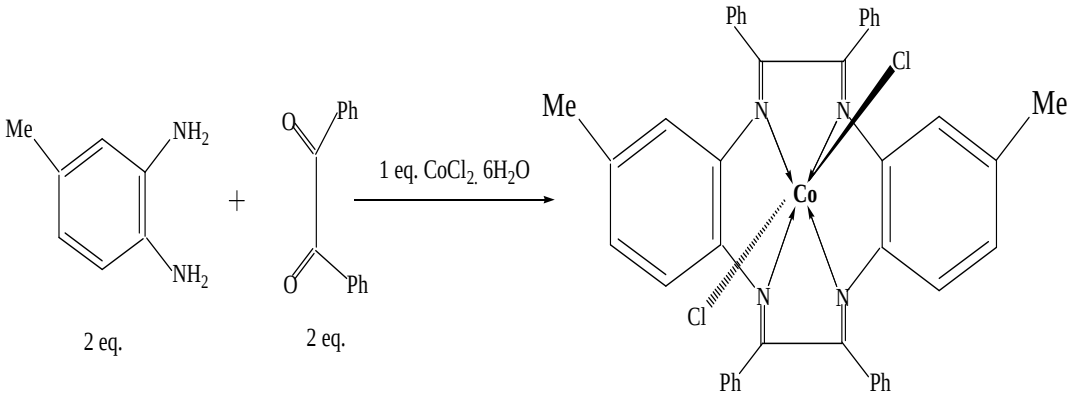


Fig. 2: The synthesis scheme of Dimethyl-dibenzo[b,h]tetraphenyl-2, 3, 8, 9-tetraazacyclododeca-1,3,7,9-tetraene macrocyclic complexes of Co(II) ($[CoC_{42}H_{32}N_4Cl_2]$) [1b]

Table 1: Physical properties of [1a] and [1b] macrocyclic complexes

Complexes	Colour	Molar conductance ($ohm^{-1}cm^{-2}mol^{-1}$)	M.P. (^0C)	Mol. Wt.	C (found) (%)	H (found) (%)	N (found) (%)
[1a]	Light yellow	65	245	755	67.65 (65.21)	4.24 (3.39)	7.42 (7.16)
[1b]	Brown	30	250	722	69.81 (68.73)	4.43 (4.73)	7.75 (8.16)

RESULTS AND DISCUSSION

3.1. IR spectra

The IR spectra of the macrocyclic complexes showed an intense band near 1620-1650 cm^{-1} due to C=N group. The other characteristic bands for both macrocycles were observed in the region 1230-1370 cm^{-1}, 1050-1260 cm^{-1}, 690-890 cm^{-1} and 2930-3000 cm^{-1} which may be assigned for the vibrations of phenyl rings and C-H stretching of methyl groups respectively [17]. The other absorption characteristics appeared in the region 520-490 cm^{-1} which may be assigned for ν(M-N) vibrations. The IR data for these macrocyclic complexes is given in **table 2.**

Table 2: IR data of both [1a] and [1b] macrocyclic complexes

Macrocyclic complexes	>C=N str.	C=C str. (Aromatic)	C—H str. (Methyl)	M—N str.
[1a]	1645	1235, 1125, 780	2935	495
[1b]	1630	1355, 1230,865	2985	515

3.2. Electronic spectral studies:

The electronic studies of these macrocyclic complexes were carried out in DMF. The observed data for the macrocycles are given in the following **table 3.** On the basis of observed results, the octahedral geometry has been assigned for both macrocycles of Fe(III) and Co(II). The ligand field parameters studies for both macrocyclic complexes (D_q, $B^{'}$ and β) have been calculated with the help of Orgel energy and Tanabe-Sugano diagrams using ν_3/ν_1 and ν_2/ν_1 ratio [18]. The nephelauxetic parameter (β) was also calculated using the relation. $\beta = B^{'} / B$ where the values of B for free metal ions are taken as Fe (III) = 1153 cm^{-1} and Co(II) = 971 cm^{-1} [19].

Table 3: Electronic spectral data for both [1a] and [1b] macrocyclic complexes

Complexes	Electronic Spectra		Stereochemistry	Ligand Field Parameter			
	Energy (cm^{-1})	Transition		D_q (cm^{-1})	ν_3/ν_1	$B^{'}$ (cm^{-1})	β
[1a]	2647	$^5T_{2g}(D) \rightarrow {}^5E_g(D)$	Octahedral	1705	1.2	1125	0.76
[1b]	16335 15246 26235	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ $^4T_{1g}(F) \rightarrow {}^4A_{2g}$ $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$	Octahedral	985	1.6	987	0.74

3.3. Mass Spectra:

The mass spectra of [1a] macrocyclic complex showed a molecular ion peak (M+1) at m/z 756 and other peaks at m/z 684 and 669 which may be attributed to the cleavage of CH_3 and Cl units respectively. [1b] macrocyclic complex showed a intense molecular ion peak (M+1) at m/z 723 and other peaks at m/z 651 and 636 which are assignable to the cleavage of CH_3 and Cl units respectively [20].

3.4. Electrochemical studies:

Electrochemical studies of [1a] and [1b] macrocyclic complexes have been carried out in DMF containing 0.1M TEAP as supporting electrolyte at a scan rate of 200 mVs^{-1}. Cyclic voltammograms of [1a] and [1b] macrocyclic complexes are shown in **fig. 3**. Diffusion coefficient (D_0) and heterogeneous electron transfer rate constant (K^0) for [1a] and [1b] macrocyclic complexes have been calculated with the help of Nicholson and Kochi methodologies [21-24].

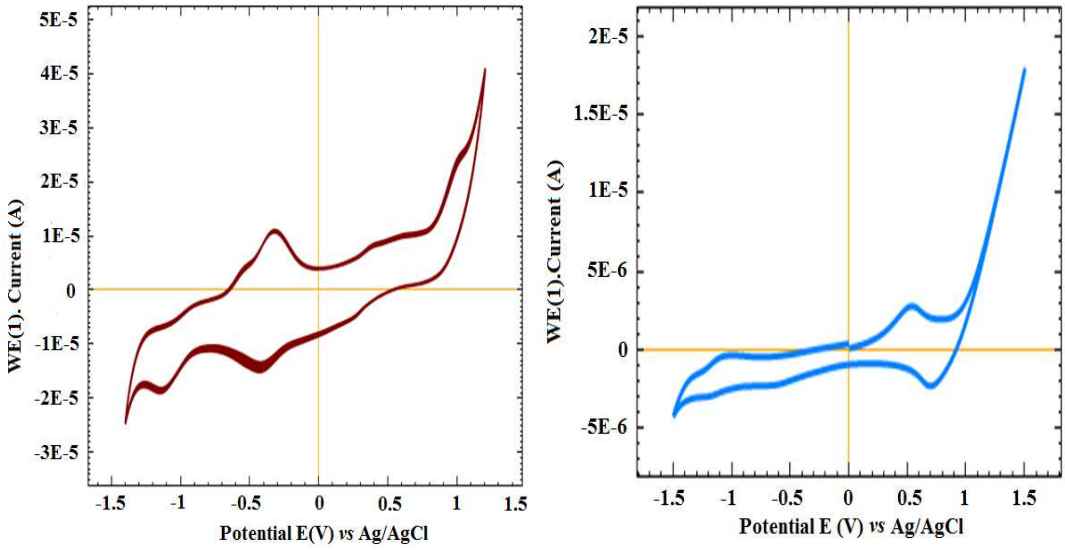
The cyclic voltammogram (**Fig. 3a**) of [1a] macrocyclic complex was recorded at 200 mVs^{-1} scan rate exhibited two anodic peaks corresponding to the cathodic peak and one anodic peak without corresponding cathodic peak. The first redox process of [1a] macrocyclic complex is a quasirreversible redox couple Fe^{+2}/Fe^{+1} corresponds to peak separation ΔE = 0.17 V and formal potential $E_{1/2}$ = -0.56 V which is also supported by the peak current ratio ($i_{pa}/i_{pc} \approx 1$). The second quasirreversible redox process is observed with formal potential $E_{1/2}$ = 0.65 V and peak separation ΔE = 0.15 V.

The cyclic voltammogram (**Fig. 3b**) of [1b] macrocyclic complex showed a reversible redox couple L/L^{-1} corresponding to peak separation ΔE = -0.13 V and formal potential ($E_{1/2}$) -1.01 V. The reversibility of this couple is also supported by the peak current ratio ($i_{pa}/i_{pc} \approx 1$) while Co^{+2}/Co^{+3} redox couple is found at formal potential +0.56 V with peak separation ΔE = 0.14 V. This redox process is the quasirreversible as also indicated by the peak current ratio i_{pa}/i_{pc}. The redox process, Co^{+2}/Co^{+1} of this macrocyclic complex is also found the quasirreversible redox process, supported by corresponding peak separation ΔE and the peak current ratio. Further, the plots of i_p against $v^{1/2}$ were found to be linear, indicating that the redox process was controlled by diffusion, following the Randles-Sevcik equation for reversible electrochemical reactions.

$$\boldsymbol{i = 2.69 \times 10^5\ n^{3/2} A D^{1/2} C v^{1/2}}$$

Where n is the number of electrons transferred in redox process, A is the area of the electrode surface (cm^2), D is the diffusion coefficient (cm^2/s), C is the concentration of analyte (M) and v is the scan rate (Vs^{-1}).

The theoretical values of diffusion coefficient (D_0) and heterogeneous electron transfer rate constant (K^0) were calculated at 200 mVs^{-1} scan rate using Nicholson and Kochi methods which were found in the decreasing order $D_0^{[1b]}$ (2.01×10^{-5} cm^2/s) > $D_0^{[1a]}$ (1.58×10^{-5} cm^2/s) and $K^0_{[1b]}$ (5.19×10^{-3} cm/s) > $K^0_{[1a]}$ (4.09×10^{-3} cm/s).



(a) **(b)**

Fig. 3: Cyclic voltammograms of (a) [1a] and (b) [1b] macrocyclic complexes recorded in DMF (1×10^{-3} M) containing 0.1 M TEAP solution as supporting electrolyte at a scan rate of 200 mVs^{-1}

3.5. Biological activity:

The antimicrobial activities of [1a] and [1b] macrocyclic complexes were studied by agar well diffusion method. The microbial cultures for these macrocyclic complexes were adjusted to 0.5 McFarland standards, which were visually comparable to a microbial pathogen suspension of approximately 1.5×10^8 cfu/ ml. 25 ml of agar media were poured into four petri plates and was swabbed with 100 µl microbial inoculums of the test microorganisms and kept for 30 minutes. A 6 mm well was cut at the centre of each agar plates and were filled with macrocyclic complexes [25-26]. DMF was used as solvent medium which was used as a negative control where as media with Gentamycin used as positive control. After 24 h of incubation at 37°C each plates were observed for the measurement of diameter at inhibition zone and in case for fungus, inhibition zone was measured after 48 h of incubation at 28°C. Antimicrobial activity for both macrocyclic complexes were evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (Hi Antibiotic Zone Scale). [27-28]

The [1b] macrocyclic complex showed maximum zone of inhibition against *E. coli* (21 mm) followed by *S. aureus* (16 mm), *B. subtilis* (16 mm) and *P. aeruginosa* (19 mm). [1a] macrocyclic complex exhibit maximum zone of inhibition against *E. coli* (20 mm) followed by *B. subtilis* (17 mm) and *S. aureus* (18 mm), *P. aeruginosa* (17 mm). Against fungal pathogen *C. albicans*, [1a] macrocyclic complex was found most effective (18 mm) followed by the [1b] macrocyclic complex (16 mm) as showed in **fig. 4**.

Table 4: Antimicrobial activity of [1a] and [1b] macrocyclic complexes

Macrocyclic complexes	Diameter of inhibition zone (mm)				
	E. coli	***P. aeruginosa***	***B. subtilis***	***S. aureus***	***C. albicans***
[1a]	20	17	17	18	18
[1b]	21	19	16	16	16
Gentamycin	24	24	24	24	20

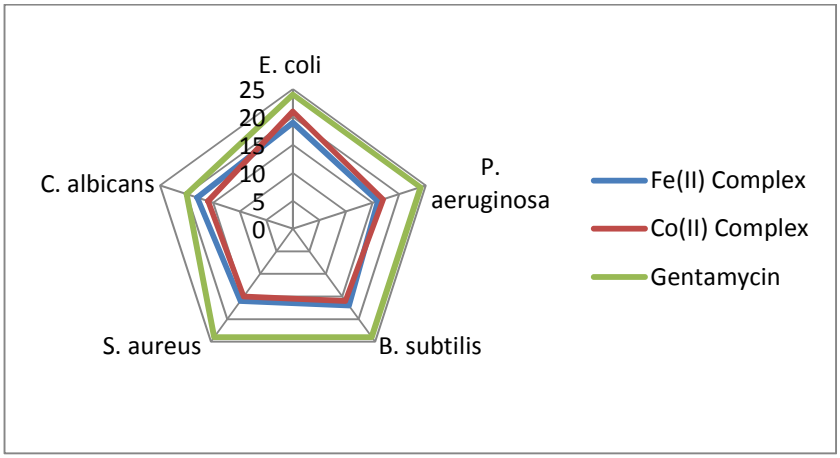


Fig. 4: Graphical presentation of antimicrobial activity of the macrocyclic complexes against pathogens

The antimicrobial activity of these macrocyclic complexes has been observed due to azomethine groups which coordinate with metal ions. Difference in the zone inhibition of macrocyclic complexes depends on the impermeability of microbial cells or on the difference in Ribosome of microbial cells [29]. The increase in antibacterial activity with the increases in the concentration of macrocyclic complexes on normal cell has been observed. This effect of metal chelates on increase in the antibacterial activity can be explained on the basis of chelation theory [30]. On chelating, the polarity of the metal ion reduced to a greater extent due to overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups. The delocalization of π-electrons enhances liphophilicity as a result penetration of the macrocyclic complexes take place on the site of enzymes. These macrocyclic complexes also hinder the respiration process of cells and block the synthesis of proteins that restricts further growth of organism [31-32].

CONCLUSION

In this communication, tetraazamacrocyclic complexes of Fe(III) and Co(II) have been synthesized by the template method using 3,4-diaminotoluene and benzil. The spectral studies have confirmed the octahedral geometry for both macrocyclic complexes. Cyclic voltammetric studies showed the interesting results for their unusual oxidation states. Heterogeneous rate constant were also calculated and were found in the order $K^0_{[1b]} > K^0_{[1a]}$. These macrocyclic complexes have shown good promise for antimicrobial activity against Gram +ve and Gram –ve bacteria.

Acknowledgments
The authors are thankful to CSIR New Delhi for financial support in the past projects and support is also acknowledged for completion the studies from SAIF Panjab University Chandigarh.

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