



Template Synthesis, Characterization and Biological Activity of Macrocyclic Transition Metal Complexes

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A novel macrocyclic transition metals complexes MLX_2 [M = Co(II), Ni(II), Cu(II) or Zn(II); X = NO_3^- , Cl^- , CH_3COO^- ; L= condensation product of 1,3-dibenzoyl benzene and 1,8-diaminonaphthlene/1,4-diaminobutane] have been prepared by template method. These complexes were characterized by spectrochemical techniques (IR, NMR), molar conductivity, magnetic moment measurements, electronic spectral data, elemental analysis, ESR and TGA studies. Their biological activities expressed in terms of MIC have been explored for Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and phytopathogenic fungi (*Candida albicans*, *Saccharomyces cerevisiae*) and compared with the standard (ciprofloxacin, amphotericine-B).

Keywords: Schiff base, Template condensation, Minimum inhibitory concentration, 1,3-Dibenzoyl benzene.

INTRODUCTION

Synthetic macrocyclic complexes are of considerable interest as they mimic some naturally occurring macrocycles [1] therefore such materials can possibly be used either as models for biological systems or precursors for the development of such materials. Condensation of dicarbonyl with diamines in the presence of metal ions to form 2 + 2 macrocyclic Schiff base metal complexes is a consequence of the coordination template effect. We have incorporated the transition metals onto macrocycles/acycles by both template and non-template methods [2-4]. In continuation of the same the present paper is concerned with the condensation of 1,3-dibenzoyl benzene and 1,8-diaminonaphthlene/1,4-diaminobutane to form macrocycle in the presence of metal ions.

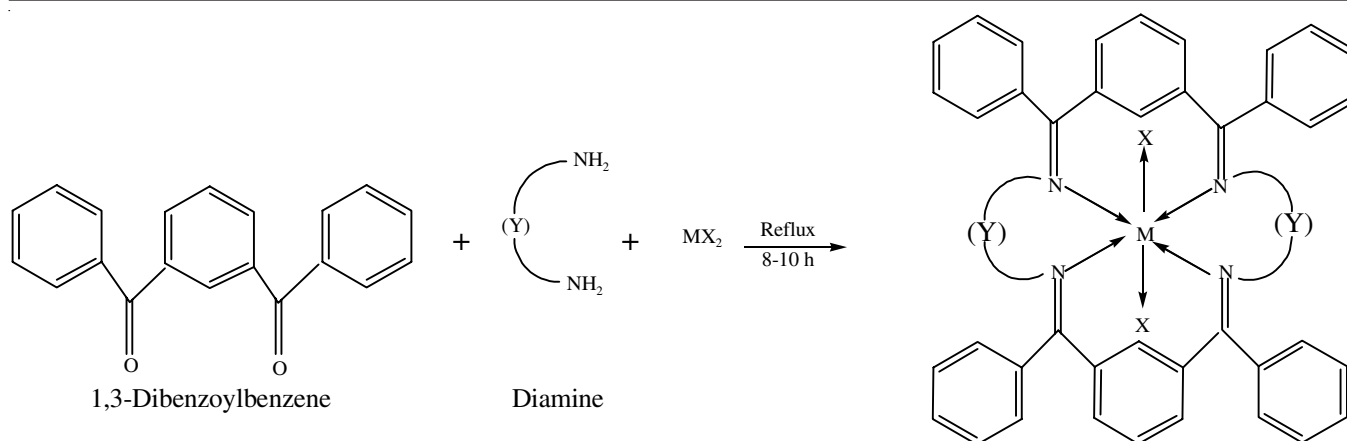
EXPERIMENTAL

Synthesis of metal complexes: All the metal complexes were prepared by template method. 1,8-Diamino naphthalene/1,4-diaminobutane (10 mmol) was dissolved in hot methanol. The corresponding Cu(II), Ni(II), Co(II), Zn(II) salts (5 mmol) dissolved in methanol were added to hot diammine solution and refluxed for 0.5 h. After that, 10 mmol of 1,3-dibenzoyl-benzene dissolved in hot methanol was added. This mixture was refluxed for 6-8 h and then concentrated to half of its volume while heating on water bath. Resulting solution was

cooled to room temperature to precipitate out the coloured solid complexes. These precipitates were filtered and washed with methanol then with acetone and diethyl ether and dried *in vacuo* (**Scheme-I**).

Biological studies: Clinical strains of human pathogenic bacteria such as *Escherichia coli* (MTCC1652), *Pseudomonas aeruginosa* (MTCC741), *Saccharomyces cerevisiae* (MTCC170), *Candida albicans* (MTCC3017) and fungi *Bacillus subtilis* (MTCC121) and *Staphylococcus aureus* (MTCC96), have been procured from Microbial Type Culture Collection IMTECH, Chandigarh. Bacterial strains are sub cultured on Nutrient Agar and yeast on malt extract agar medium. These are incubated aerobically at 37 °C.

Antimicrobial activity: The antimicrobial activity of all the metal complexes have been determined against four bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* and two yeast strains *Candida albicans* and *Saccharomyces cerevisiae* using the agar well diffusion method [5]. Density of all the microbial cultures were adjusted to 0.5 McFarland standards, which are visually com-parable to a microbial suspension of approximately 1.5×10^8 cfu/mL. 20 mL of the Nutrient agar medium (for bacteria) and Malt extract agar medium (for yeast) has been poured into each Petri plate. One hundred microlitre inocula of the test microorganisms has been spread in each plate and kept for 15 min for adsorption. Solutions of each complex have



Scheme-I: Synthesis of complexes

been prepared by dissolving the complex in 20 % DMSO. Using sterile cork borer of 8 mm diameter, wells are bored into the seeded agar plates and these are loaded with a 100 μL volume of the solution of each complex. All the plates are incubated at 37 $^\circ\text{C}$ for 24 h. Antimicrobial activity of each complex has been evaluated by measuring the zone of growth inhibition against the test organisms with a zone reader (HiAntibiotic zone scale). Ciprofloxacin and amphotericin are used as a positive control. This procedure is performed in three replicate plates for each organism.

Determination of minimum inhibitory concentration (MIC): Minimum inhibitory concentration of the complexes for each test organism has been determined by following the modified agar well diffusion method. A twofold serial dilution of each complex has been prepared. Each complex has been dissolved in 20 % DMSO to achieve a concentration of 100 mg/mL followed by dilution in sterile distilled water (1:1) to achieve a decreasing concentration range of 50 to 0.39 mg/mL. A 100 μL volume of each dilution has introduced into wells (in triplicate) in the agar plates already seeded with 100 μL of standardized inoculum (10^6 cfu/mL) of the test microbial strain. All test plates are incubated aerobically at 37 $^\circ\text{C}$ for 24 h and observed for the inhibition zones. Zone of inhibition (> 8 mm) has been observed in each plate. Concentration of the complex that completely inhibited the growth of the microorganism has been taken as minimum inhibitory concentration.

RESULTS AND DISCUSSION

All the complexes were insoluble in water and common organic solvents but fairly soluble in dimethyl sulphoxide and dimethyl formamide. The physico-chemical characteristic data is given in Table-1.

Infrared: Absence of a pair of medium intensity peak at 3300 cm^{-1} shows that there is no N-H stretching vibrations which indicates the absence of N-H bond of diamine. Strong absorption band at 1655 cm^{-1} corresponding to $>\text{C}=\text{O}$ group of dibenzoylbenzene is absent which shows the reaction between carbonyl group of 1,3-dibenzoylbenzene and diamine [6]. Further appearance of strong absorption band near 1567 cm^{-1} shows formation of macrocyclic Schiff base. These bands are due to $\nu(\text{C}=\text{N})$ stretching vibrations [7]. As lone pair of electrons of nitrogen are drifted towards metal atom so $\nu(\text{C}=\text{N})$ stretching comes at lower value. This also shows that metal coordinates with nitrogen of $(\text{C}=\text{N})$ group [8]. Bands near 1341, 1256 cm^{-1} are due to C-N stretching, medium intensity band near 3255, 3044 cm^{-1} and are due to C-H stretching of aromatic ring. While peaks at 2920, 2960 and 2851 cm^{-1} are due to C-H stretching of aliphatic carbon, when diamine is diaminobutane. Bands near 715, 714 are due to $\nu(\text{C}-\text{H})$ out of plane bending vibration. Bands near 619, 623, 670 cm^{-1} is due to $\nu(\text{COO}^-)$ as asymmetric stretching of acetate ion. Metal complexes shows a band in far IR region at 495 and 430 cm^{-1} which correspond to $\nu(\text{M}-\text{N})$ vibration. These bands show

TABLE-1
ANALYTICAL DATA OF METAL COMPLEXES DERIVED FROM
1,3-DIBENZOYL BENZENE AND 1,8-DIAMINONAPHTHALENE/1,4-DIAMINO BUTANE

Compd. No.	m.f.	Colour	Yield (%)	m.w.	Elemental analysis (%): Found (calcd.)			
					M	C	H	N
1	$[\text{C}_{60}\text{H}_{40}\text{N}_4\text{Cu}(\text{OAc})_2]$	Black	60	997.5	6.05 (6.36)	72.01 (72.18)	3.76 (4.01)	5.15 (5.61)
2	$[\text{C}_{60}\text{H}_{40}\text{N}_4\text{ZnCl}_2]$	White	65	952.4	6.51 (6.87)	75.22 (75.67)	4.02 (4.20)	5.36 (5.88)
3	$[\text{C}_{60}\text{H}_{40}\text{N}_4\text{NiCl}_2]$	Black	54	945.7	5.92 (6.13)	76.03 (76.21)	4.07 (4.23)	5.65 (5.92)
4	$[\text{C}_{60}\text{H}_{40}\text{N}_4\text{Co}(\text{OAc})_2]$	Black	52	992.9	5.54 (5.93)	72.11 (72.51)	3.72 (4.02)	5.30 (5.64)
5	$[\text{C}_{48}\text{H}_{44}\text{N}_4\text{Cu}(\text{OAc})_2]$	Dark brown	50	857.5	7.11 (7.40)	66.71 (67.17)	5.02 (5.13)	6.15 (6.53)
6	$[\text{C}_{48}\text{H}_{44}\text{N}_4\text{ZnCl}_2]$	White	50	812.4	7.86 (8.06)	70.56 (70.98)	5.13 (5.42)	6.52 (6.90)
7	$[\text{C}_{48}\text{H}_{44}\text{N}_4\text{NiCl}_2]$	Light green	48	805.7	7.03 (7.29)	71.24 (71.57)	5.02 (5.46)	6.70 (6.95)
8	$[\text{C}_{48}\text{H}_{44}\text{N}_4\text{Co}(\text{OAc})_2]$	Green	45	852.9	6.62 (6.90)	67.23 (67.53)	5.03 (5.15)	6.28 (6.56)

coordination of azomethine nitrogen to the central metal atom.

NMR: The ^1H NMR spectrum of zinc complex of 1,8-diaminonaphthalene and 1,3-dibenzoylbenzene shows peaks at δ 5.4 and multiplet at δ 6.5 to 7.07. These all peaks are due to aromatic protons. Peak due to N-H proton is missing which shows the absence of hydrogen with nitrogen and formation of C=N bond. The ^1H NMR spectrum of zinc complexes of 1,4-diaminobutane and 1,3-dibenzoylbenzene shows a peak at δ 8.8 due to aromatic protons and at δ 2.1 to δ 2.7 due to aliphatic CH_2 of diaminobutane. Peak present in the spectrum of 1,4-diaminobutane at δ 1.74 N-H is absent in the spectrum of complex which again confirms the formation of C=N bond.

Magnetic measurements and electronic spectral studies: The magnetic moments of copper complexes lie in the range of 1.74-1.84 B.M. This value suggests distorted octahedral geometry of single electron system. Distorted octahedral geometry was further confirmed by electronic spectra which exhibit bands near 17450-19650 and 14400-16250 cm^{-1} due to $^2\text{B}_{1g} \rightarrow ^2\text{B}_{2g}$, $^2\text{B}_{1g} \rightarrow ^2\text{E}_g$, $^2\text{B}_{1g} \rightarrow ^2\text{A}_{2g}$ transitions.

The magnetic moment 2.82-2.92 B.M. and electronic spectra of nickel(II) complexes confirm octahedral geometry of two unpaired electron system. Bands near 16560-17050 and 26850-28240 cm^{-1} were assigned to $^3\text{A}_{2g} \rightarrow ^3\text{T}_{1g}(\text{F})$, $^3\text{A}_{2g} \rightarrow ^3\text{T}_{1g}(\text{P})$ transitions.

The effective magnetic moment of cobalt(II) complexes (4.84-4.95) B.M. is in agreement with the three unpaired electrons and octahedral geometry. Electronic spectra also confirm D_{4h} symmetry of high spin cobalt(II) complexes. In electronic spectra absorption bands at 8100-9250, 12100-15850 and 18200-20550 cm^{-1} may be assigned to $^4\text{T}_{1g} \rightarrow ^4\text{T}_{2g}(\text{F})$, $^4\text{T}_{1g} \rightarrow ^4\text{A}_{2g}(\text{F})$, $^4\text{T}_{1g} \rightarrow ^4\text{T}_{2g}(\text{P})$ transitions.

ESR: The ESR spectra of Cu(II) complexes has been recorded at room temperature and liquid nitrogen temperature (LNT) in solid state on X-band at 9.1 GHz frequency under the magnetic field of 3000 G. The ESR spectrum of 1,8-diaminonaphthalene and 1,3-dibenzoylbenzene at LNT exhibits a single isotropic peak. The analysis of the spectrum gives g_{iso} value 2.09 which is greater than 2.0023. This g value greater than 2.0023 is because of mixing spin orbital coupling of metal orbitals involved in the molecular orbitals containing the unpaired electron with the filled ligand orbitals. This shows

that the complex has unpaired electron localized in $d_{x^2-y^2}$ orbital of the Cu(II) ion and concludes that there is distortion in Cu(II) complex from octahedral geometry that is from O_h symmetry to D_{4h} symmetry. The observed g values suggest that d-shell is more than half filled in these Cu(II) complexes. The deviation of g value from the free electron value (2.0023) may be due to interaction between the spin and orbital motion of electron.

The ESR spectrum of complex 1,4-diaminobutane and 1,3-dibenzoylbenzene at LNT shows sharp peaks. The analysis of the peaks gives g_{\parallel} value 2.12 and g_{\perp} value 2.03. As $g_{\parallel} > 2.0023$ so the complex has unpaired electron localized in $d_{x^2-y^2}$ orbital and has distorted octahedral geometry. The observed g_{\parallel} value for copper complex is less than 2.3 shows covalent character of metal ligand bond [9]. $G = (g_{\parallel} - 2.0023)/(g_{\perp} - 2.0023)$ measures the exchange interaction in crystalline solid. The value of G for these complexes is found to be 4.24 which are more than 4. This shows that there are less exchange interactions in solid state.

Thermal analysis: TGA-DTA analysis of metal complexes was done in air atmosphere at a heating rate of 10 $^{\circ}\text{C}/\text{min}$ over a temperature range of about 1000 $^{\circ}\text{C}$ using alumina as reference. Thermo gravimetric analysis shows that these complexes exhibit high thermal stability. Moisture is removed up to temperature 100 $^{\circ}\text{C}$ [10]. Acetate ion is removed up to temperature of 250 $^{\circ}\text{C}$ and organic moiety is removed at 322 $^{\circ}\text{C}$.

The initial weight loss of 6.7 % up to temp 97.5 $^{\circ}\text{C}$ may be due to loss of moisture. A weight loss of 3.3 % at 205 $^{\circ}\text{C}$ may be due to loss of acetate ion. There is a decomposition curve in the range of 205-322 $^{\circ}\text{C}$ with 73.4 % weight loss may be due to loss of organic moiety. The residue left is 16.6 % which is due to formation of Cu_2O .

Pharmacological effect of complexes: Prepared macrocyclic Schiff base complexes were tested for four human pathogenic bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* and two yeast strains *Candida albicans* and *Saccharomyces cerevisiae*. Complex **3** is active for Gram-positive bacteria, Gram-negative bacteria and yeast. Complex **4** is active only for *Bacillus subtilis* (Gram-positive bacteria) and yeast. Complex **5** shows activity only for Gram-negative bacteria and complex **7** for *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* complex **6** do not show any activity for yeast *Candida albicans*. While complexes **1**, **2**, **8** do not show any activity (Tables 2 and 3).

TABLE-2
in vitro ANTIMICROBIAL ACTIVITY OF SYNTHETIC CHEMICAL COMPOUNDS THROUGH AGAR WELL DIFFUSION METHOD

Compd. No.	Diameter of growth of inhibition zone (mm) ^a					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Saccharomyces cerevisiae</i>
1	–	–	–	–	–	–
2	–	–	–	–	–	–
3	25	22	18	22	20	18
4	20	–	–	–	12	16
5	–	–	10	26	–	–
6	22	27	22	18	–	18
7	23	18	12	–	–	–
8	–	–	–	–	–	–
Ciprofloxacin	24.0	26.6	25.0	22	–	–
Amphotericin-B	–	–	–	–	16.6	19.3

– : No activity; ^aValues, including diameter of the well (8 mm), are means of three replicates

TABLE-3
MIC OF MACROCYCLIC SCHIFF BASE COMPLEXES (mg/mL)

Compd. No.	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Saccharomyces cerevisiae</i>
1	–	–	–	–	–	–
2	–	–	–	–	–	–
3	12.5	12.5	25	25	25	50
4	25	–	–	–	nt	50
5	–	–	nt	6.25	–	–
6	12.5	6.25	12.5	25	–	25
7	12.5	50	nt	–	–	–
8	–	–	–	–	–	–
Ciprofloxacin	6.25	6.25	6.25	12.5	–	–
Amphotericin-B	–	–	–	–	12.5	12.5

nt – not tested; – no activity

Minimum inhibitory concentration is determined only for the complexes which show *in vitro* activity. Complex **3** shows minimum inhibitory concentration very close to standard antibiotic ciprofloxacin and amphotericin-B. Complex **5** also shows comparable MIC for Gram-negative bacteria *Pseudomonas aeruginosa*. Complex **6** shows same MIC for *Staphylococcus aureus* as ciprofloxacin and comparable MIC for other strains. MIC of complexes whose activity is very less has not been determined.

Conclusion

Based on infrared, NMR, ESR and TGA data it is concluded that these metal complexes have distorted octahedral geometry. Thermal studies show that these metal complexes are thermally stable. Infrared spectra reveal that the complexes are tetradentate with N donor atoms. These complexes are biologically active against Gram-positive bacteria, Gram-negative bacteria and phytopathogenic fungi. Minimum inhibitory concentrations of these complexes are comparable with standard antibiotics ciprofloxacin and amphotericin-B.

Complexes **5** and **6** show good activity and further research may be continued to explore their use in living beings.

REFERENCES

1. M. Shakir, S. Khatoon, S. Parveen and Y. Azim, *Transition Met. Chem.*, **32**, 42 (2007).
2. R. Malhotra, S. Kumar, Jyoti, H.R. Singal and K.S. Dhindsa, *Indian J. Chem.*, **39A**, 421 (2000).
3. S. Kumar, R. Malhotra and K.S. Dhindsa, *Polyhedron*, **11**, 1383 (1992).
4. S. Singh, R. Malhotra, A. Hooda and K.S. Dhindsa, *Bull. Soc. Chim. Belg.*, **105**, (1996).
5. K.R. Aneja, C. Sharma and R. Joshi, *JJundishapur J. Microbiol.*, **4**, 175 (2011).
6. Q. Zeng, J. Sun, S. Gou, K. Zhou, J. Fang and H. Chen, *Transition Met. Chem.*, **23**, 371 (1998).
7. L.K. Gupta and S. Chandra, *Transition Met. Chem.*, **31**, 368 (2006).
8. C. Lodeiro, R. Bastida, E. Bertolo, A. Macias and A. Rodriguez, *Transition Met. Chem.*, **28**, 388 (2003).
9. K. Sharma, Parveen, D.P. Singh and R. Chopra, *Der Pharma Chemica*, **7**, 292 (2015).
10. R.H. Satpute, M.A. Sakhare, A.O. Dhokte and B.R. Arbad, *Arch. Appl. Sci. Res.*, **7**, 31 (2015).