Studies of Transition Metal Complexes and Their Antibacterial Activities

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Abstract: Tetraaza Macrocyclic complexes of transition metals of Ni (II), Cu(II), Cr(III), Fe(III), Mn(II) were synthesized in methanolic media by template method. The complexes were characterized by elemental analysis, UV-Vis, Infrared spectroscopy. *In vitro* antibacterial activity of macrocyclic complexes against five bacteria i.e. *Streptococcus mutans*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* were tested to assess their inhibiting the activities and compared with standard with ampicilline. [Journal of American Science 2010; 6(9):559-564]. (ISSN: 1545-1003).

Keywords: Tetraaza macrocyclic complexes, template synthesis, antibacterial activity

Introduction:

Now- a- days macrocyclic chemistry is a growing area of research in inorganic and bioinorganic chemistry in view of its biological significance. The studies of macrocycles have undergone tremendous growth in recent years and their complexation chemistry with a wide variety of metal ions has been extensively studied. Macrocyclic complexes are considered to mimic the synthetic models of metalloporphyrins and metallocorrins due to their intrinsic structural properties. (Mruthyunjayaswamy *et. al.,* 2005; Shankar *et. al.,* 2009). Efforts have been made to achieve peripheral substitution, the appended substituents providing points of attachment for further structural modifications. As a consequence, these substituents might create the possibility of synthesis of more complex compounds serving as new biologically important models.(Kubaszewski and Malinski 1992; Eilmes 1985) These compounds have also been received considerable attention due to their possible applications in medicine.(Vaum *et. al.,* 1982; Caemelbecke *et. al.,* 2005).

Design and synthesis of synthetic model compounds that mimic the physical and chemical properties of the active sites present in metalloenzymes is very essential and the studies on such compounds is becoming increasingly important in understanding biological functions of these macrocycles.(Fenton and Okawa 1993; Karabocek *et. al.,* 2006; Kimura 1993; Cunha *et. al.,* 2005; Blain *et. al.,* 1990; Singh *et. al.,* 2010)

Macrocyclic complexes have also received special attention because of their mixed soft–hard donor character and versatile coordination behaviour and their pharmacological properties, *i.e*., toxicity against bacterial growth (Chandra *et. al.,* 2006; Collen *et. al.,* 1997; Rosu *et. al.,* 2006; Filho *et. al.,* 1998; Turhan-Zitouni *et. al.,* 2001; Chandra *et. al.,* 2009).

Antibiotics are vital medicinally important molecules used for the treatment of bacterial infections in both human and animals but due to the regular used of antibiotic, resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem (Guillemot, 1999). One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors as macrocyclic complexes. Keeping the importance of the macrocyclic complexes and their antibacterial activity, the present article has been taken into account.

Experimental

Materials

All chemicals used in this study were of AnalaR grade. Five macrocyclic complexes were prepared and characterized.

Preparation of macrocyclic complexes (General procedure)

All complexes were synthesized by the template method according to the literature (Rafat *et. al.,* 2004), by condensation of ethylenediamine and acetone/acetyl acetone in the presence of respective metal salts (chloride, sulphate). To a methanolic

solvent (≈50 ml), ethylenediamine, acetone/acetyl acetone followed by metal salt in the ratio 2:2:1 were added in round bottom flask. Shake well and refluxing was carried out for 6-8 hours. The change in colour was appeared. The round bottom flask was kept aside for its cooling .The filteration and washing were carried out by methanol and dried in vacuum. The coloured complexes were obtained and taken for further studies.

Macrocyclic complexes-

(A) 1,4,8,11-tetraaza 5,7,7,12,14,14-hexamethyl tetradeca 4,11- diene N(II) macrocyclic complex
(B) 1,4,8,11-tetraaza 5,7,7,12,14,14-hexamethyl tetradeca 4,11- diene Cu(II) macrocyclic complex
(C) 1,4,8,11-tetraaza 5,7,712,14,14-hexamethyl tetradeca 4,11- diene Cr(III) macrocyclic complex
(D) 1,4,8,11-tetraaza 5,7,12,14-tetramethyl tetradeca 4,11- diene Fe(III) macrocyclic complex
(E) 1,4,8,11-tetraaza 5,7,12,14-tetramethyl tetradeca 4,11- diene Mn(II) macrocyclic complex

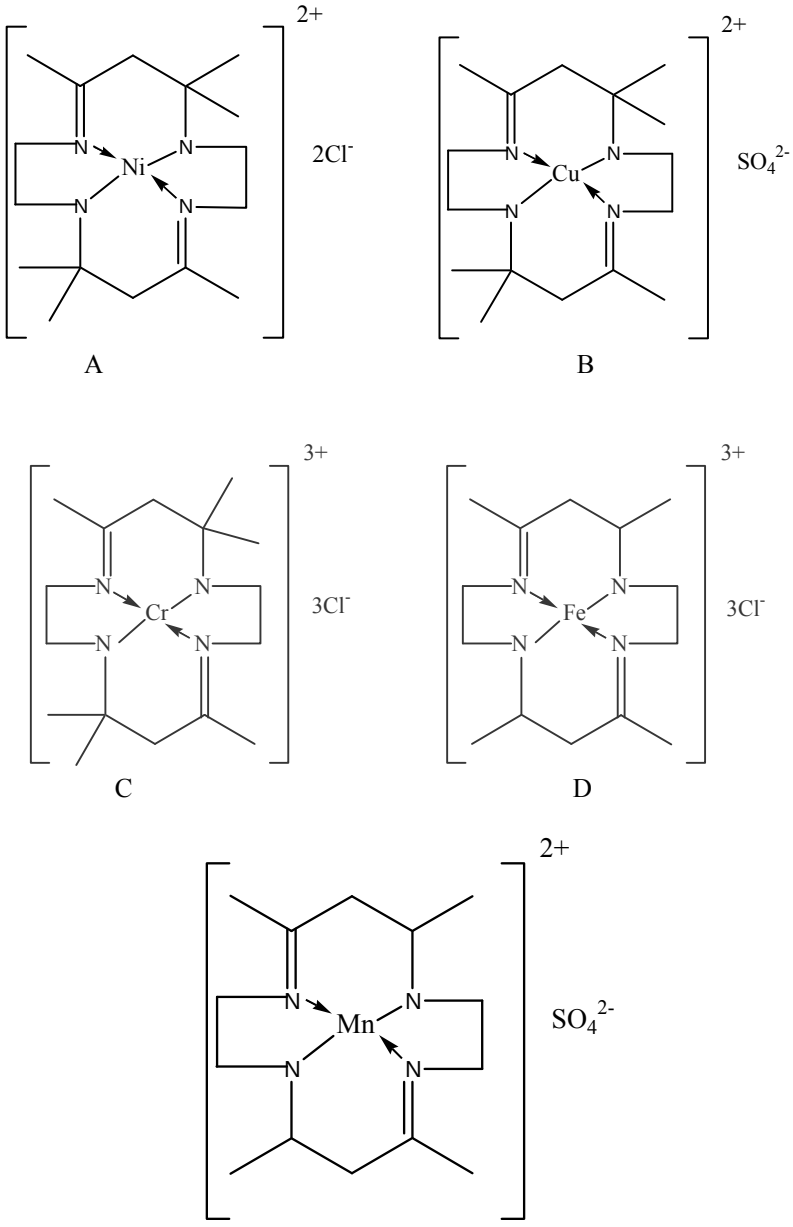

Figure-1, The proposed structure of the complexes

Preparation of samples: Samples were prepared by dissolving macrocyclic complexes in appropriate solvent (methanol or water).

Culture media: Muller Hinton agar media M-173 (Hi media Pvt. Ltd., Mumbai, India) was used for conducting antibacterial tests.

Mi croorganisms used **:** 5 different pathogenic bacteria such as *Streptococcus mutans* (MTCC 890) , *Escherichia coli* (MTCC 226) , *Staphylococcus aureus* (MTCC 1144), *Streptococcus pyogenes* (MTCC 655), *Streptococcus pneumoniae* (MTCC 422) were used. All the strains were grown and maintained on nutrient agar slants at 4^{o}C.

Antimicrobial assay:

The cup- plate method was used to evaluate the antibacterial activity (Prabhat *et. al.,* 2005). This method depends upon the diffusion of the tested material to such an extent that growth of added microorganisms is prevented entirely in a zone around the hole containing a solution of tested material. One hundred microlitres of diluted inoculums of 10^5CFU/ml of 24hours old cultures of test organisms were mixed in Muller Hinton agar medium and shaken. Then media was poured (25-30ml) in sterilized Petri dishes (20 × 90 mm). Wells of 6 mm diameter were punched into the agar medium and filled with 45µl of synthesized complexes (100mg/ml). All the solvents served as negative control. Antibiotic (ampicilline concentration 100mg/ml.) was simultaneously used as positive control. Each sample was assayed in triplicate and the mean values were observed. The plates were then incubated at 37^{0}Cfor 24 h. The antimicrobial activity was interpreted from the size of the diameter of zone of inhibition measured in millimetres (mm), it was observed as the clear zones surrounding the hole evaluated by measuring the inhibition zone diameter. The inhibition of the bacterial growth expressed in percentage terms was determined from the growth in the test plate relative to the respective control plate as given below:

Percentage of potency = (C-T) 100/C

Where, C=diameter of the bacterial growth in the control

T= diameter of the bacterial growth in the test

Results and Discussion

The present studies described the new macrocyclic complexes of different metals synthesis. These complexes were crystalline solids, non-hygroscopic. The UV-Vis measurements were carried out using a spectrophotometer (systronics UV-Vis spectrophotometer 117).The formulae for these macrocyclic complexes can be assigned on the basis of analytical data (Table-1). Analytical and spectroscopic data (Table-2) enable us to predict the possible structure of the synthesized complexes. (Fig-1).

The I.R. spectral data indicates some important assignments. The complexes exhibit a C=N absorption in the range of 1600-1616 cm^{-1}, which together with the absence of C=O absorption around 1700 cm^{-1}. It shows that amino group of ethylenediamine was condensed with the carbonyl group of acetylacetone/acetone to give a cyclic structure. In the complexes, N-H band were observed at 3263-3279 cm^{-1} indicates the coordination to the metal through nitrogen of NH group. Bands appearing in the 2911-2945 cm^{-1} region corresponds to -CH. Bands at 1378-1381 cm^{-1} show the presence of two methyl groups at same carbon.

Antibacterial activity

The antibacterial activity of macrocyclic complexes against pathogens at concentration 100mg/ml are given in Table 3. Inhibition of solvent (water or methanol) was found to be very negligible and taken as zero mm. All these synthesized macrocyclic complexes showed good antibacterial activity (figure2).The antibacterial activity of complexes were observed in increasing order Cu< Mn< Cr< Fe<Ni

The maximum zone of inhibition against *S. pneumoniae* ie 18mm was observed in Cr complex and the minimum zone of inhibition in Cu and Fe complexes against *E.coli* ie 8 mm. Similarly Ni showed maximum antibacterial activity against *S. mutans*(18 mm) and minimum against *E. coli* (11mm).

The present study has shown that these macrocyclic complexes are potentially rich source of antibacterial agents. This demonstrates their importance in tranditional remedies in the population. Each macrocyclic complexes (100 mg/ml) were used for determination of their potency against tested pathogens and compared with antibiotic (Table.4). All the complexes have good antibacterial activity but Ni showed strong activity.

Further, on the basis of chelation theory, antibacterial activity of the metal chelates can be explained. Chelation may enhance the biochemical potential of bioactive species. Because on chelation, the polarity of the metal ion will be reduced due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups.

Hence macrocyclic complexes become very stable due to delocalization of π-electrons. It enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms. (Raman *et. al.*, 2008).

Table 1. λmax, elemental analysis of macrocyclic complexes

Macrocyclic complexes/ (Molecular Formula)	λ max (nm)	Elemental Analysis							
		C%		H%		N%		M%	
		Cal	Found	Cal	Found	Cal	Found	Cal	Found
$NiC_{16}H_{30}N_4Cl_2$	572	47.09	46.98	7.36	7.32	13.73	13.62	14.39	14.29
$CuC_{16}H_{30}N_4SO_4$	464	43.89	43.15	6.85	6.77	12.80	12.59	14.51	14.34
$CrC_{16}H_{30}N_4Cl_3$	440	43.98	43.92	6.87	6.34	12.82	12.76	11.91	11.48
$FeC_{14}H_{26}N_4Cl_3$	220	40.72	40.59	6.30	6.23	13.57	13.25	13.57	13.14
$MnC_{14}H_{26}N_4SO_4$	490	41.90	41.67	6.48	6.10	13.97	13.26	13.70	13.35

Table 2. Infrared spectral data of macrocyclic complexes (cm^{-1})

Macrocyclic complexes	C=N	NH	-CH	$C(CH_3)_2$
$NiC_{16}H_{30}N_4Cl_2$	1600	3269	2916	1380
$CuC_{16}H_{30}N_4SO_4$	1615	3275	2921	1378
$CrC_{16}H_{30}N_4Cl_3$	1600	3263	2945	1381
$FeC_{14}H_{26}N_4Cl_3$	1616	3268	2911	-
$MnC_{14}H_{26}N_4SO_4$	1605	3279	2911	-

Table 3. Antibacterial activity of synthesized complexes(conc. 100 mg/ml)

Macrocyclic Complex	*Streptococcus* mutans	*E.coli*	*Staphylococcus* aureus	*Streptococcus* pyogenes	*Streptococcus* pneumoniae
Ni M.C.	18	11	12	15	14
Cu M.C.	11	8	15	14	13
Cr M.C.	12	9	11	13	18
Fe M.C.	13	8	13	15	16
Mn M.C.	17	10	10	13	12
Antibiotic (Ampicilline)	19	19	18	19	20

Diameter of inhibition Zone (mm)

Table 4. Percentage of potency of macrocyclic complexes compared with antibiotic(Ampicilline)

Macrocyclic Complex	*Streptococcus mutans*	*E.coli*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Streptococcus pneumoniae*
Ni M.C.	5.26	42.11	33.33	21.05	30
Cu M.C.	42.11	57.89	16.67	26.32	35
Cr M.C.	36.84	52.63	38.89	31.58	10
Fe M.C.	31.58	57.89	27.78	21.05	20
Mn M.C.	10.53	47.37	44.44	31.58	40

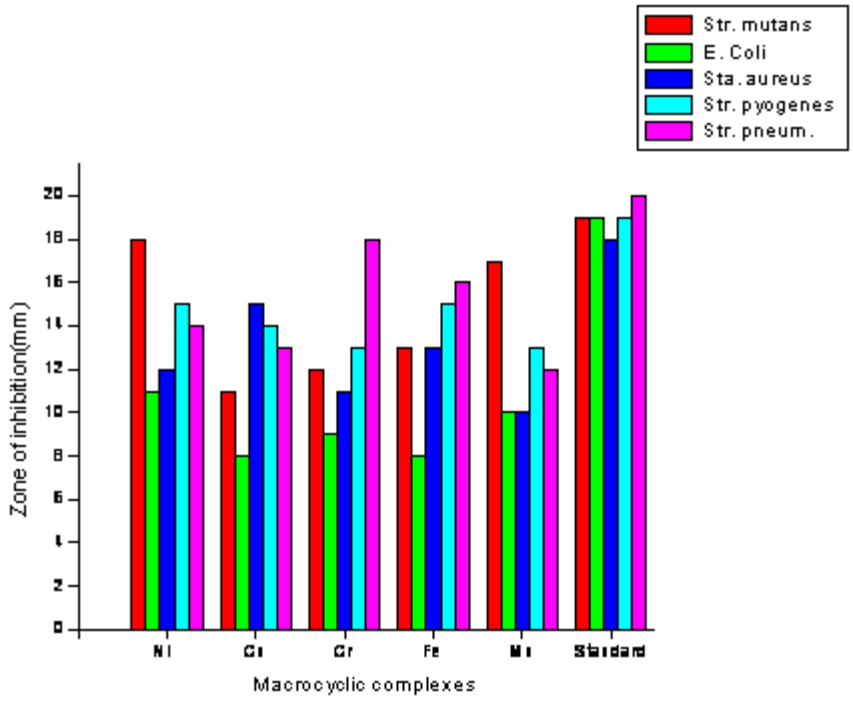

Figure 2. Antibacterial activity of macrocyclic complexes

Conclusion

This study is a preliminary evaluation of antibacterial activity of macrocyclic complexes. It indicates that the complexes have the potential to generate new antimicrobial metabolites. The macrocyclic complexes demonstrating antibacterial activity could result in the discovery of new chemical classes of antibiotics that could serve as selective agent for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases.

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