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Synthesis and Spectral and Antibacterial Studies of Bivalent Transition Metal Ion Macrocyclic Complexes[1]

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Abstract—A new series of the macrocyclic complexes of type $[M(C_{18}H_{16}N_4O_2)X_2]$, where M = Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and X = Cl^-, NO_3^-, CH_3COO^-, has been synthesized by the condensation of succinyldihydrazide with benzil in the presence of bivalent metal ions. The complexes have been characterized with the aid of elemental analyses, conductance measurements, and electronic, NMR, and infrared spectral studies. On the basis of these studies, a six-coordinate distorted octahedral geometry in which two nitrogen and two carbonyl oxygen atoms are suitably placed for coordination toward metal ion has been proposed for all the complexes. The complexes were tested for their in vitro antibacterial activity. Some of the complexes showed remarkable antibacterial activity against some selected bacterial strains.

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INTRODUCTION

Research on diverse aspects of new macrocyclic compounds has evoked considerable worldwide interest in recent years. The condensation reaction between diketones and primary diamines in the presence of metal ion has played a vital role in the development of macrocyclic complexes (**MCC**). MCC are thermodynamically more stable and more selective ion bindes than open-chain analogs. The multifarious role is played by the naturally occurring macrocycles in biological systems. The chemistry of synthetic MCC is also of great importance due to their use as dyes and pigments, MRI contrast agents, and models for naturally occurring macrocycles [1–4]. Macrocyclic nickel complexes find use in DNA recognition and oxidation [5], while macrocyclic copper complexes find use in DNA-binding and cleavage [6]. Some MCC have been reported as showing antibacterial, antifungal, and anti-inflammatory activities [7–9]. Macrocyclic metal chelating agents are useful to detect tumor lesions [10]. Prompted by these, in the present paper a new series of macrocyclic complexes of Co(II), Ni(II), Cu(II), Zn(II), and Cd(II) obtained by the template condensation reaction of succinyldihydrazide and benzil has been reported. The complexes have been characterized with the help of various physicochemical techniques like elemental analyses, IR, NMR, magnetic susceptibilities, electronic spectra, and molar conductance. These macrocyclic complexes were also screened for their in vitro antibacterial activity.

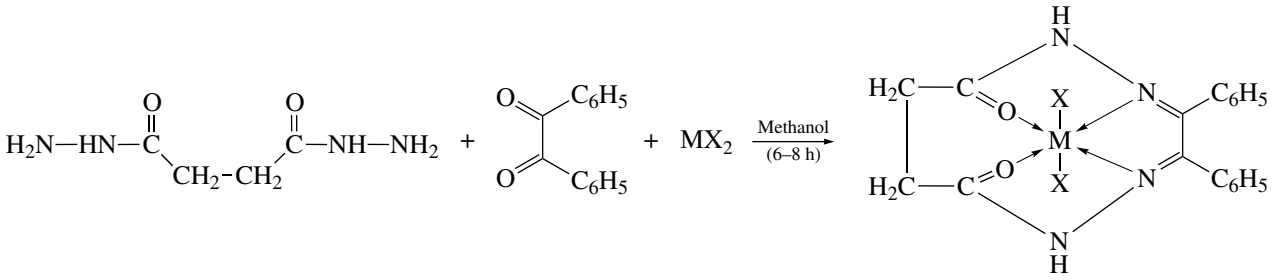
[1] The article is published in the original.

EXPERIMENTAL

Synthesis of complexes. All the reported MCC were prepared by the template method. To a stirring methanolic solution (~50 cm^3) of succinyldihydrazide (10 mmol) was added bivalent cobalt, nickel, copper, zinc, and cadmium salts (10 mmol) dissolved in a minimum quantity of methanol (20 cm^3). The resulting solution was refluxed for 0.5 h. After that benzil (10 mmol) dissolved in ~20 cm^3 of methanol was added, and the mixture was again refluxed for 6–8 h. On overnight cooling light colored complexes were formed, which were filtered, washed with methanol, acetone, and ether, and dried in vacuo (the yield was 65%). The complexes were soluble in DMF and DMSO but insoluble in common organic solvents and water. They were found to be thermally stable up to ~250°C and then decomposed.

The template syntheses of the complexes may be represented by the scheme.

Analytical and physical measurements. The microanalyses of C, H, and N were carried out at the Sophisticated Analytical Instrument Facility (CDRI, Lucknow). The metal contents were determined by standard EDTA methods. Electronic spectra (DMF) were recorded on a Cary 14 spectrophotometer. The magnetic susceptibility measurements were carried at the IIT Roorkee. The IR spectra were recorded on a infrared spectrophotometer in the range 4000–200 cm^{-1} using Nujol mulls. The NMR spectra were recorded on a Bruker NMR spectrometer (300 MHz). The conductivity was measured on a digital conductivity meter (HPG System, G-3001).



where M = Co(II), X = Cl^- (I), Cu(II), X = Cl^- (V),
Co(II), X = NO_3^- (II), Cu(II), X = NO_3^- (VI),
Co(II), X = CH_3COO^- (III), Zn(II), X = CH_3COO^- (VII),
Ni(II), X = CH_3COO^- (IV), Cd(II), X = CH_3COO^- (VIII).

Scheme

Biological assay. Some of the synthesized macrocyclic complexes were tested for in vitro antibacterial activity against some bacterial strains using spot-on-lawn on Muller Hinton Agar [11].

Four test pathogenic bacterial strains viz., *Bacillus cereus* (**MTCC** 1272), where MTCC – Microbiol Type Culture Collection, *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 739) and *Staphylococcus aureus* (MTCC 1144), were considered for determination of minimum inhibitory concentration (**MIC**) of selected complexes.

The test pathogens were subcultured aerobically using Brain Heart Infusion Agar (HiMedia, Mumbai, India) at 37°C/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) an organism was grown overnight in 10 ml BHI broth, and centrifuged at 5.000 *g* for 10 min and the pellet was suspended in 10 ml of a phosphate saline buffer (PBS, pH 7.2). The optical density at 545 nm (OD-545) was adjusted to obtain 10^8 cfu/ml followed by plating serial dilution onto plate count agar (HiMedia, Mumbai, India).

The MIC is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation. Antimicrobial activity of the compounds was evaluated using spot-on-lawn on Muller Hinton Agar (**MHA**, HiMedia, Mumbai, India). Soft agar was prepared by adding 0.75% agar in Muller Hinton Broth (HiMedia, Mumbai, India). Soft agar was inoculated with 1% of 10^8 cfu/ml of the test pathogen and 10 ml was overlaid on MHA. From 1000X solution of the compound (1 mg/ml of DMSO) 1, 2, 4, 8, 16, 32, 64, and 128X solutions were prepared. Dilutions of standard antibiotics (Linezolid and Cefaclor) were also prepared in the same manner: 5 µl of the appropriate dilution was spotted on the soft agar and incubated at 37°C for 24 h. The zone of inhibition of compounds as considered after subtraction of the inhibition zone of DMSO. Negative control (with no compound) was also observed.

RESULTS AND DISCUSSION

The analytical data suggest the formula of macrocyclic complexes as $[M(C_{18}H_{16}N_4O_2)X_2]$, where M = Co(II), Ni(II), Cu(II), Zn(II), and Cd(II) and X = Cl^-, NO_3^-, and CH_3COO^-. The test for anions is positive after decomposing the complexes with concentrated HNO_3, indicating their presence inside the coordination sphere. Conductivity measurements in DMSO indicate them to be nonelectrolytic in nature [12] (10–20 Ohm^{-1} cm^2 mol^{-1}). All compounds give satisfactory elemental analyses results as shown in Table 1.

A close perusal of IR spectra exhibits a pair of strong bands at ~3200 and ~3250 cm^{-1} corresponding to ν(N–H), which is present in the spectrum of succinyldihydrazide but absent in the spectra of all the complexes [13]. However, a broad peak at ~3350–3400 cm^{-1} observed in the spectra of all the complexes is due to ν(NH) stretching vibrations [14, 15]. A strong peak at ~1665 cm^{-1} in the spectrum of succinyldihydrazide is attributed to the CO group of the CONH moiety. This peak is shifted to a lower frequency (~1625–1640 cm^{-1}) in the spectra of all the complexes [16], suggesting the coordination of oxygen of the carbonyl group with the metal. Further no strong absorption band was observed near 1690 cm^{-1} in the IR spectra of the complexes as observed in the spectrum of benzil. This indicates the absence of the >C=O group of the benzil moiety. This confirms the condensation of the carbonyl group of benzil and the amino group of succinyldihydrazide [17, 18]. This fact is also supported by the appearance of a new strong absorption band in the region ~1590–1610 cm^{-1}, which may be attributed to ν(C=N) stretching vibrations [19, 20]. These results provide strong evidence for the formation of macrocyclic frame [21]. The lower values of ν(C=N) indicate coordination of azomethine nitrogen to metal [22]. The bands present at ~1350–1000 cm^{-1} may be assigned due to the ν(C–N)

Table 1. Analytical data of the bivalent cobalt, nickel, copper, zinc, and cadmium complexes derived from succinyldihydrazide and benzil

Complex	Contents (found/calcd), %				Color	F.w.
	C	H	N	M		
$[Co(C_{18}H_{16}N_4O_2)Cl_2]$ (**I**)	48.19/48.00	3.73/3.55	12.52/12.44	13.16/13.11	Bluish green	450
$[Co(C_{18}H_{16}N_4O_2)(NO_3)_2]$ (**II**)	42.80/42.94	3.13/3.18	16.95/16.69	11.79/11.72	Orange	503
$[Co(C_{18}H_{16}N_4O_2)(OAc)_2]$ (**III**)	53.19/53.11	4.19/4.42	11.29/11.26	11.89/11.87	Dark red	497
$[Ni(C_{18}H_{16}N_4O_2)(OAc)_2]$ (**IV**)	53.31/53.22	4.33/4.43	11.27/11.29	11.27/11.69	Dark gray	496
$[Cu(C_{18}H_{16}N_4O_2)Cl_2]$ (**V**)	46.86/46.70	3.59/3.52	12.35/12.32	13.69/13.97	Bluish green	455
$[Cu(C_{18}H_{16}N_4O_2)(NO_3)_2]$ (**VI**)	42.69/42.56	3.25/3.15	16.64/16.55	12.62/12.51	Brown	507
$[Zn(C_{18}H_{16}N_4O_2)(OAc)_2]$ (**VII**)	52.51/52.48	4.23/4.37	11.27/11.13	12.99/12.92	White	503
$[Cd(C_{18}H_{16}N_4O_2)(OAc)_2]$ (**VI-II**)	47.88/47.96	3.93/3.99	10.10/10.17	20.56/20.42	Off white	550

vibration. The bands present at ~2900–3130 cm^{-1} may be assigned to ν(C–H) vibrations of the benzil moiety and methylene moiety. The far-infrared spectra show bands in the region ~420–460 cm^{-1} corresponding to ν(M–N) vibrations in all the complexes [23–25]. The presence of bands in all the complexes in the region ~420–460 cm^{-1} originates from (M–N) azomethine vibration modes and gives an idea about coordination of azomethine nitrogen [26]. The bands present at ~300–310 cm^{-1} may be assigned to ν(M–Cl) vibrations [23, 25]. The bands present at ~210–230 cm^{-1} in all nitrato complexes are assignable to ν(M–O) vibrations of the nitrato group [23].

The ^1H NMR spectrum of the zinc(II) complex shows a broad singlet at 8.45 ppm due to protons of the CONH moiety [14, 27]. A singlet peak at 2.43 ppm may be due to CH_2 protons [28]. The multiplets in the region 6.9–7.5 ppm may be assigned to aromatic protons [29].

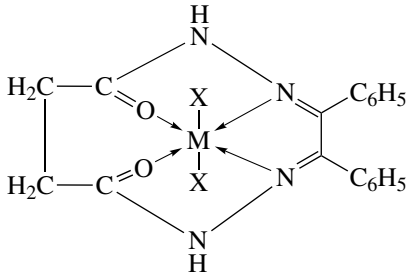
The magnetic moments of the cobalt complexes at room temperature were found to be in a range of 4.87–4.92 μ_B. These data correspond to three unpaired electrons. The electronic spectra of the cobalt complexes show bands at ~8110–9000 (ν_1), 12400–15450 (ν_2), and 18700–20220 cm^{-1} (ν_3), respectively. The spectral data resemble to those reported to be octahedral [30]. Thus, assuming the effective symmetry to be D_{4h}, the various bands can be assigned to $^4T_{1g} \longrightarrow {}^4T_{2g}(F)$, ($\nu_1$), $^4T_{1g} \longrightarrow {}^4A_{2g}(F)$, ($\nu_2$), and $^4T_{1g} \longrightarrow {}^4T_{1g}(P)$, ($\nu_3$), respectively. It appears that the symmetry of these complexes is not ideally octahedral but is D_{4h}. The assignment of the first spin-allowed band seems plausible, since the first band appears approximately at half an energy of the visible band [31].

The nickel complexes show magnetic moments in the 2.85–2.88 μ_B range at room temperature showing an octahedral environment around the divalent nickel ion in all complexes. The solution spectra of the nickel complexes exhibit a well discernable band with a shoulder on the low-energy side. The other two bands observed in the region at ~16760–17030 (ν_2), and 26800–28100 cm^{-1} (ν_3), are assigned to $^3A_{2g} \longrightarrow {}^3T_{1g}(F)$ (ν_2), and $^3A_{2g} \longrightarrow {}^3T_{1g}(P)$ (ν_3), respectively. The first two bands result from the splitting of one band ν_1, and are in the range at ~9700–10200 and 11800–12500 cm^{-1}, which can be assigned to $^3B_{1g} \longrightarrow {}^3E_g$ and $^3B_{1g} \longrightarrow {}^3B_{2g}$, assuming the effective symmetry to be D_{4h} (component of $^3T_{2g}$ in O_h symmetry) [31]. The intense higher-energy band at ~34550 cm^{-1} may be due to a π–π* transition of the (C=N) group. Various bands do not follow any regular pattern and seem to be anion-independent. The spectra are consistent with the distorted octahedral nature of these complexes.

The magnetic moments of the copper complexes lie in the range 1.77–1.79 μ_B. The electronic spectra of the copper complexes exhibit bands in the region ~17550–19500 cm^{-1} with a shoulder on the low-energy side at ~14500–16200 cm^{-1} and show that these complexes are distorted octahedral [30, 31]. Assuming tetragonal distortion in the molecule, the *d*-orbital energy level sequence for these complexes may be $x^2 - y^2 > z^2 > xy > xz > yz$ and the shoulder can be assigned to $z^2 \longrightarrow x^2 -$

y^2 ($^2B_{1g} \longrightarrow {}^2B_{2g}$) and the broad band contains both $xy \longrightarrow x^2 - y^2$ ($^2B_{1g} \longrightarrow {}^2E_g$) and xz, $yz \longrightarrow x^2 - y^2$ ($^2B_{1g} \longrightarrow {}^2A_{2g}$) transitions [32]. The band separation of the spectra of the complexes is about 2520 cm^{-1}, which is consistent with the proposed geometry of the complexes [32]. Therefore, it may be concluded that all the copper complexes are distorted octahedral.

Based on elemental analyses, conductivity, magnetic, electronic, NMR, and IR spectral studies, the structure may be proposed for all the complexes:



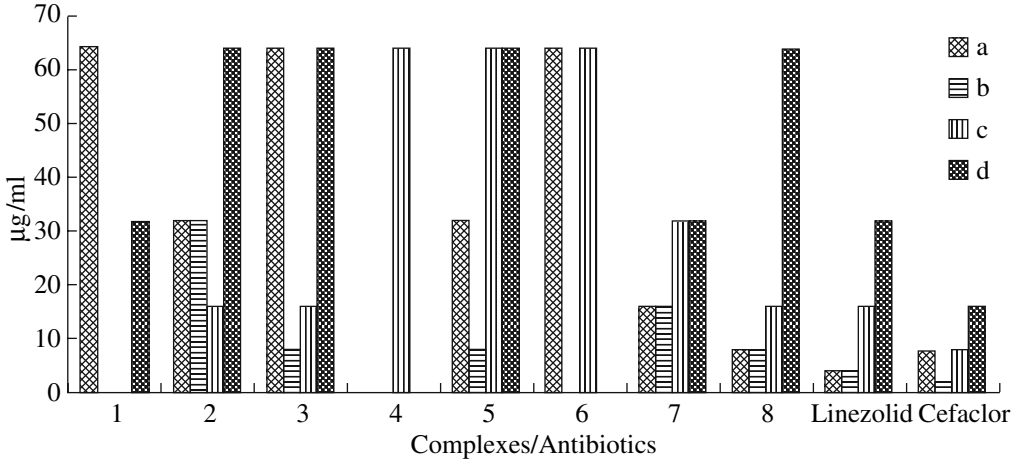
where M = Co(II), Ni(II), Cu(II), Zn(II), Cd(II);
X = Cl^-, NO_3^-, CH_3COO^-.

The synthesized macrocyclic complexes were tested for their in vitro antibacterial activity against four test bacteria *Bacillus cereus* (MTCC 1272), *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 739), and *Staphylococcus aureus* (MTCC 1144), the MIC shown by the complexes against these bacterial strains was compared with MIC shown by standard antibiotics *Linezolid* and *Cefaclor* (Table 2, figure). Complex **I** showed remarkable MIC only against *Salmonella typhi*. It showed a MIC of 32 μg/ml against bacterial strain *Salmonella typhi* (MTCC 733), which is equal to MIC shown by standard antibiotic *Linezolid* against the same bacterial strain. The MIC of complexes **II**, **III**, and **VIII** against *Escherichia coli* (MTCC 739) was found to be 16 μg/ml, which is equal to the minimum inhibitory concentration shown by standard antibiotic *Linezolid* against the same bacterial strain. Complex **VII** showed

Table 2. Minimum inhibitory concentration (MIC) shown by the complexes against test bacteria by using agar dilution assay

Complex	MIC (μg/ml)*			
	a	b	c	d
$[Co(C_{18}H_{16}N_4O_2)Cl_2]$ (**I**)	64	128	>128	32
$[Co(C_{18}H_{16}N_4O_2)(NO_3)_2]$ (**II**)	32	32	16	64
$[Co(C_{18}H_{16}N_4O_2)(OAc)_2]$ (**III**)	64	8	16	64
$[Ni(C_{18}H_{16}N_4O_2)(OAc)_2]$ (**IV**)	128		64	128
$[Cu(C_{18}H_{16}N_4O_2)Cl_2]$ (**V**)	32	8	64	64
$[Cu(C_{18}H_{16}N_4O_2)(NO_3)_2]$ (**VI**)	64	128	64	
$[Zn(C_{18}H_{16}N_4O_2)(OAc)_2]$ (**VII**)	16	16	32	32
$[Cd(C_{18}H_{16}N_4O_2)(OAc)_2]$ (**VIII**)	8	8	16	64
Cefaclor	8	2	8	16
Linezolid	4	4	16	32

* a – *Bacillus cereus* (MTCC 1272); b – *Staphylococcus aureus* (MTCC 1144); c – *Escherichia coli* (MTCC 739); d – *Salmonella typhi* (MTCC 733). Cefaclor and Linezolid are standard antibiotics.



Comparison of MIC of the complexes with standard antibiotics up to a concentration of 64 μg/ml: a – *Bacillus cereus* (MTCC 1272), b – *Staphylococcus aureus* (MTCC 1144), c – *Escherichia coli* (MTCC 739), d – *Salmonella typhi* (MTCC 733). Cefaclor and Linezolid are standard antibiotics

a MIC of 32 μg/ml against bacterial strain *Salmonella typhi* (MTCC 733), which is equal to MIC shown by standard antibiotic *Linezolid* against the same bacterial strain. Complex **VIII** shows a minimum inhibitory concentration of 8 μg/ml against bacterial strain *Bacillus cereus* (MTCC 1272), which is equal to MIC shown by standard antibiotic *Cefaclor* against the same bacterial strain. Among the series under testing for determination of the minimum inhibitory concentration, complex **II** was found to be most potent complex as showing MIC equal to that of standard antibiotic Cefaclor and Linezolid against *Bacillus cereus* (MTCC 1272) and *Escherichia coli* (MTCC 739), respectively. However, complexes **IV** and **VI** showed poor antibacterial activity or no activity against all bacterial strains among the whole series (Table 2, figure).

Thus, based on elemental analyses, conductivity, magnetic, electronic, NMR, and IR spectral studies, the same structure may be proposed for all complexes.

It has been suggested that chelation/coordination reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with the donor group within the whole chelate ring system. This process of chelation thus increases the lipophilic nature of the central metal atom, which, in turn, favors its permeation through the lipoid layer of the membrane thus causing the metal complex to cross the bacterial membrane more efficiently thus increasing the activity of the complexes. In addition, from this many other factors, such as solubility, dipole moment, and conductivity, influenced by metal ion may be possible reasons for remarkable antibacterial activities of these complexes [33–36]. It also has been 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 the biological utilization ratio and activity of the complexes [37–39].

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