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ANTIOXIDANT, ANTIMICROBIAL ACTIVITIES, AND CHARACTERIZATION OF METAL COMPLEXES DERIVED FROM BENZILDIHYDRAZONE AND 3-METHYL 2,4PENTANEDIONE

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ABSTRACT

In this study, metal complexes were synthesized by the reaction of benzildihydrazone and 3methyl 2,4pentanedione and the complexes formulated as :[M($C_{40}H_{40}N_{8}$)X_2], where M= Cu(II) and Co(II) and X=NO_3^-, Cl^- andCH3COO$^-$. The novel molecule was characterized by FT-IR and UV-VIS, spectra in addition of the elemental analysis. The free radical scavenging ability of the complexes was determined by it interaction with the stable-free radical 2,2"-diphenyl-1-picrylhydrazyl (DPPH) . The results obtained against the DPPH radical confirmed that the complexes are more effective to arrest the formation of the DPPH radicals and the lower IC_{50} values observed in antioxidant assays demonstrate that these complexes exhibited differential and selective effects to scavenge radicals and hence the potential to eliminate the radicals .The synthesized macrocyclic complexes were tested for in vitro antibacterial activity against four test pathogenic bacterial strains viz Bacillus cereus (MTCC 1272), Salmonella typhi (MTCC 733), Escherichia coli (MTCC 739) and Staphylococcus aureus (MTCC 1144). Antibacterial activities exhibited by the synthesized macrocyclic complexes were found significant than commercially available antibiotics Linezolid and Cefaclor. The process of chelation increases the lipophilicity of metal complexes, metal complexes penetrate the bacterial membrane(lipid membrane) more effectively thus increases the inhibition potential of the complexes.

Keywords: Bacillus Cereus (MTCC 1272), Benzildihydrazone, DPPH, Escherichia Coli (MTCC 739) 3methyl 2,4pentanedione, Salmonella Typhi (MTCC 733), And Staphylococcus Aureus (MTCC 1144).

I. INTRODUCTION

There has been a growing interest in the study of macrocyclic complexes and in the synthesis of coordination compounds due to their potential application in the areas such as MRI, imaging with isotopes and radiotherapy, luminescent probes and DNA cleaver . The synthesis and study of macrocycles have undergone a tremendous growth and their complexation chemistry with a wide variety of metal ions has been extensively studied. Macrocyclic ligands are polydentate ligands containing their donor atoms either incorporated in or, less commonly, attached to a cyclic backbone [1-5]. The chemistry of Schiff-base is an important field in coordination chemistry due to their ability to react with a range of metal ions to form stable complexes which

have applications in different fields like biomedical , biomimetic, catalytic systems . A number of Schiff-base complexes have been used as oxygen carriers to mimic complicated biological systems . Furthermore, metal complexes of chromium, manganese, nickel, copper, zinc, and ruthenium with a wide variety of Schiff-bases are have been used as catalysts for carbonylation, hydrogenation, hydroformylation, and epoxidation reactions [6-9]. Ligands containing donor atoms like N, O, S show broad biological activity and are of special interest because of the variety of ways in which they are bonded to metal ions. [10-11].]. Schiff bases having chelation with oxygen, nitrogen donors and their complexes have been used as drugs because of wide variety of biological activities against bacteria, fungi, and tumors [12-15]

II. EXPERIMENTAL

All the chemicals used were of Anal R grade, and Metal salts were purchased from Himediaand HPLC. benzil, hydrazine hydrate and 3-Methyl 2, 4 pentanedione were purchased from Sigma Aldrich were used as received. All solvents used were of standard/spectroscopic grade.

2.1 Preparation of Macrocyclic Complexes

All the complexes were synthesized by template method. To a stirring methanolic solution (~50cm3) of benzildihydrazone (~10 m mol) was added divalent, manganese, iron, nickel and copper salt (~5m mol) dissolved in minimum quantity of methanol (~20 cm3). The resulting solution was refluxed for half an hour. After that 3methyl 2,4pentanedione (10 mmol) dissolved in methanol (~20cm3) added in the refluxing mixture and again refluxed for 6-8h, depending upon the metal salt . The complexes were filtered, washed with Methanol, Acetone and then dreied in vacuum desiccator (Yield 35%55%). The purity of the complexes was checked by TLC. The complexes were found soluble in DMF and DMSO, but were insoluble in common organic solvents and water. They were thermally stable up to ~250oC and then decomposed.

The template syntheses of the complexes derived from benzildihydrazone and 3-methyl 2,4-pentanedione may be represented by the following scheme (1):

$$2\,C_{14}H_{14}N_4 + 2\,C_6H_{10}O_2 + MX_2 \xrightarrow{\text{METHANOL ,6-8 HOUR}} [M(C_{40}H_{40}N_8)X_2] + 4H_2O$$

Where M = Cu(II) and Co(II)

X=NO^-_3, Cl^- and CH_3COO^-

III. RESULTS AND DISCUSSION

The analytical data of divalent macrocyclic complexes derived from benzildihydrazone and 3-methyl 2,4-pentanedione and the complexes formulated as: :$[M(C_{40}H_{40}N_8)X_2]$,where M= Cu(II), Co(II) and X=NO^-_3, Cl^- and CH_3COO^- .All the macrocyclic complexes are dark colored solids and are soluble in dimethylformamide or dimethylsulphoxide. Conductivity measurement in dimethylsulphoxide indicated them to be non-electrolyte (10-20 $ohm^{-1}cm^2\ mol^{-1}$)[16,17]. The test for anions is positive only after decomposing the complexes, indicating their presence inside the coordination sphere. All complexes were decomposed over at ~250ºC indicating their thermal stability. The complexes have been characterized with the help of various physicochemical techniques such as infrared, electronic, magnetic susceptibility measurements, molecular weight determination. On the

basis of these studies, a six- coordinate octahedral geometry, in which two nitrogen atoms are suitably placed for coordination towards the metal ion, has been proposed for all the complexes.

Table (i): Physical properties and analytical data of Metal complexes of divalent transition metal ions derived from benzildihydrazone and 3methyl 2, 4pentanedione found (calculated).

S. N0	Complex	color	M. W.	%of C	%of H	%of N	%of M
1.	$[Co(C_{40}H_{40}N_8)Cl_2]$	green	761	63.07 (63.09)	5.25 (5.21)	14.71 (14.69)	7.75 (7.71)
2.	$[Co(C_{40}H_{40}N_8)(NO_3)_2]$	white	815	58.89 (58.90)	4.90 (4.88)	13,74 (13.73)	7.23 (7.20)
3.	$[Co(C_{40}H_{40}N_8)(OAc)_2]$	yellow	809	59.33 (59.36)	4.94 (4.90)	13.84 (13.83)	7.29 (7.30)
4.	$[Cu(C_{40}H_{40}N_8)(NO_3)_2]$	brown	883.5	54.36 (54.40)	4.53 (4.52)	12.68 (12.66)	7.41 (7. 40)
5.	$[Cu(C_{40}H_{40}N_8)(OAc)_2]$	orange	813.5	59.00 (58.99)	4.91 (4.93)	13.76 (13.77)	7.80 (7.79)

3.1 Ir Spectra

A close perusal of infrared spectra exhibit a pair of the strong band at ~3200 cm^{-1} and ~3250 cm^{-1} corresponding to $\nu(NH_2)$, is present in the spectrum of benzildihydrazone but absent in the spectra of all the complexes [18,19]**.** Further no strong absorption band was observed near~16901710 cm^{-1} as observed in spectrum of 3methyl 2,4pentanedione indicating the absence of ν(C=O) group of 3methyl 2,4pentanedione moiety. This gives an idea about the condensation reaction of carbonyl group of 3methyl 2,4pentanedione and amino group of benzildihydrazone [20,21]. This fact is further supported by the appearance of a new strong absorption band in the region ~15951615 cm^{-1} which may be attributed due to ν(C═N) [21,22]. These results provide strong evidence for the formation of macrocyclic frame [22]. The lower values of ν(C═N) indicate the coordination of azomethines nitrogen to the metal atom [22]. The bands present at ~13651000 cm^{-1} may be assigned due to ν(C=N) vibration. The bands presents at ~29153130 cm^{-1} may be assigned due to ν (CH) vibrations of benzildihydrazone and 3methyl 2,4pentanedione moiety. The far infrared spectra show bands in the region ~ 420445 cm^{-1} corresponding to ν(M-N) vibrations in all the complexes [23, 24, 25]. The presence of bands in all the complexes in region ~425445 cm^{-1} originate from ν(M-N) azomethines vibration modes and gives an idea about coordination of azomethines nitrogen [26,]. The bands present at ~310315 cm^{-1} may be assigned as being due to ν(M-Cl) vibrations [24, 25]

3.2 Magnetic Measurements and Electronic Spectral Studies Copper Complexes

The magnetic moments of copper complexes lay in the range 1.761.79 B.M. The absorption spectra of the copper complexes exhibit bands in the region ~ 17,550-19,600 cm^{-1} with a shoulder on the low energy side at ~14,560-16,100 cm^{-1}, and show that these complexes are distorted octahedral [27]. Assuming tetragonal

distortion in the molecule, the d orbital energy level sequence for these complexes may be: x2 y2> z2 > xy > xz > yz and the shoulder can be assigned to: $z_2 \rightarrow x_2\ y_2$ ($2B_{1g} \rightarrow 2B_{2g}$) and the broad band contains both the xy → $x_2\ y_2$ ($2B_{1g} \rightarrow 2Eg$) and xz, yz → $x_2\ y_2$ ($2B_{1g} \rightarrow 2A_{2g}$) transitions**.** The band separation of the spectra of the complexes is of the order 2980 cm^{-1}, which is consistent with proposed geometry of the complexes [28]. Therefore, it may be concluded that all the complexes of Cu(II) metals of this series are distorted octahedral.

3.3 Cobalt Complexes

The magnetic moment was measured at room temperature and lie in the range 4.914.94 B.M., which corresponds to three unpaired electrons. The solution spectra of cobalt(II) complexes exhibit absorption in the regions ~8100-9000 cm^{-1}(ν1), ~12500-15750 cm^{-1}(ν2) and~18700-20250 cm^{-1} (ν3), respectively. The spectra resemble to those which are reported earlier as octahedral**.** Thus assuming the effective symmetry to be D4th the various bands can be assigned to: 4T1g → 4T2g (F), (ν1), 4T1g → 4A2g (F), (ν ν2), 4T1g → 4T1g (P), (ν3), respectively. It appears that the symmetry of these complexes is not idealized Oh, but is D4h. [29,30,31]

IV. ANTIBACTERIAL ACTIVITY OF CU(II) AND CO (II) COMPLEXES DERIVED FROM BENZILDIHYDRAZONE AND 3METHYL 2,4PENTANEDIONE.

4.1 In vitro antibacterial activity

The synthesized macrocyclic complexes were tested for in vitro antibacterial activity against some bacterial strains using spot on lawn on Muller Hinton Agar by reported method.

4.2 Test Pathogens

Four test pathogenic bacterial strains viz Bacillus cereus (MTCC 1272), Salmonella typhi (MTCC 733), Escherichia coli (MTCC 739) and Staphylococcus aureus (MTCC 1144) were considered for determination of MIC (Minimum Inhibitory Concentration) of selected complexes.

4.3 Biological Results and Discussion

The MIC (minimum inhibitory concentration) shown by the complexes against these bacterial strains was compared with MIC shown by standard antibiotics Linezolid and Cefaclor (Table:2)Complex 1 showed a MIC of 8 µg/ml againsts bacterial strain Escherichia coli (MTCC 739), which is equal to MIC shown by standard antibiotic Cefaclor against the same bacterial strain. Complex 2 registered a MIC of 8µg/ml, againsts bacterial strain Bacillus cereus (MTCC 1272), which is equal to MIC shown by standard antibiotic Cefaclor against the same bacterial strain. Further complex 5 and 2 showed a minimum inhibitory concentration 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 3 showed MIC of 2 µg/ml against bacterial strain Staphylococcus aureus (MTCC 1144), which is equal to MIC shown by standard antibiotic Cefaclor against the same bacterial strain. Complex 1 registered a MIC of 4 µg/ml against bacterial strain Staphylococcus aureus (MTCC 1144), which is equal to MIC shown by standard antibiotic Linezolid against the same bacterial strain. Among the series under test for determination of MIC, complex 5 was found most potent complex (Table:ii) .

Table (ii): Minimum Inhibitory Concentration (MIC) shown by copper(II) and Co(II) complexes derived from benzildihydrazone and 3methyl 2,4pentanedione against test bacteria by using spot on l awn on Muller Hinton Agar method

S. No	Complexes	MIC (µg/ml)			
		a	**b**	**c**	**D**
1.	$[Co(C_{40}H_{40}N_8)Cl_2]$	32	32	8	>128
2.	$[Co(C_{40}H_{40}N_8)(NO_3)_2]$	8	64	64	32
3.	$[Co(C_{40}H_{40}N_8)(OAc)_2]$	64	2	2	>128
4.	$[Cu(C_{40}H_{40}N_8)(NO_3)_2]$	32	4	4	64
5.	$[Cu(C_{40}H_{40}N_8)(OAc)_2]$	32	64	64	32
6.	**Cefaclor**	**8**	**2**	**8**	**16**
7.	**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.

V. DPPH ASSAY (ANTIOXIDANT ACTIVITY)

The DPPH radical is a stable free radical commonly used as a substrate to evaluate in vitro antioxidant activity. Antioxidants can scavenge radicals by hydrogen donation, which causes a decrease in DPPH absorbance (Ancerewicz et al., 1998). Radical scavenging activity was determined by the reaction of the stable DPPH radical with the compounds, in accordance with the method of Choi et al.(2002) with some modifications. Different concentrations of compound (5–500 µM) were mixed with a methanolic solution containing the DPPH radical, resulting in a final concentration of 300 µM DPPH. The mixture was incubated for 30 min at RT, and the absorbance was measured at 517 nm. The values are expressed as the percentage of inhibition of DPPH absorbance (% inhibition) in relation to the control values without the compound, as calculated from the following equation:

I%DPPH= [(ACAS/AC) X100]

where I =DPPH inhibition,

Ac = absorbance of the control reaction mixture excluding the test compound

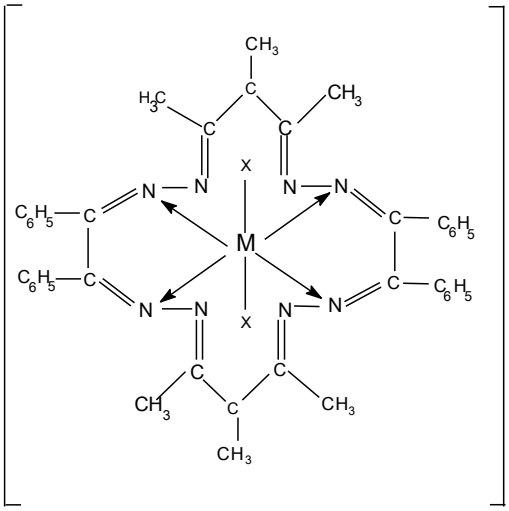
As = absorbance of the test compound in different concentrations.

Table (iii): Result of antioxidant activity of synthesized compounds

S.No	Complexes	Concentration	1% DPPH
1.	$[Co(C_{40}H_{40}N_8)Cl_2]$	5µM	41
		10µM	28
		50µM	31
		100µM	36
2.	$[Co(C_{40}H_{40}N_8)(NO_3)_2]$	5µM	29
		10µM	30
		50µM	17
		100µM	28
3.	$[Co(C_{40}H_{40}N_8)(OAc)_2]$	5µM	25
		10µM	21
		50µM	19
		100µM	ND
4.	$[Cu(C_{40}H_{40}N_8)(NO_3)_2]$	5µM	11
		10µM	21
		50µM	ND
		100µM	ND
5.	$[Cu(C_{40}H_{40}N_8)(OAc)_2]$	5µM	ND
		10µM	ND
		50µM	ND
		100µM	5

VI. CONCLUSION

Therefore based on elemental analyses, conductivity measurements, magnetic susceptibility measurements, electronic and infrared spectral studies the structure shown in figure-1 derived from benzil dihydrazone and 3-methyl 2,4 pentanedione with divalent metal salts may be proposed for all of the complexes.



Where M = Cu(II) and Co(II)

$X=NO^-_3$, Cl^- and CH_3COO^-

The synthesized macrocyclic complexes were tested for in vitro antibacterial activity against Four test pathogenic bacterial strains viz Bacillus cereus (MTCC 1272), Salmonella typhi (MTCC 733), Escherichia coli (MTCC 739) and Staphylococcus aureus (MTCC 1144). Antibacterial activities exhibited by the synthesized macrocyclic complexes were found significant than commercially available antibiotics Linezolid and Cefaclor. The process of chelation increases the lipophilicity of metal complexes,metal complexes penetrate the bacterial membrane(lipid membrane) more effectively thus increases the inhibition potential of the complexes. The results obtained against the DPPH radical confirmed that the complexes are more effective to arrest the formation of the DPPH radicals and the lowerIC50 values observed in antioxidant assays demonstrate that these complexes exhibited differential and selective effects to scavenge radicals and hence the potential to eliminate the radicals.Among the series under test for determination of minimum inhibitory concentration in Copper(II) and Cobalt(II)) complex derived from benzildihydrazone and 3 methyl 2,4pentanedione ,complex of [CoNO3)2]was found to be most potent complex as it showed MIC equal to that of standard antibiotic Cefaclor against Bacillus cereus (MTCC 1272 and Salmonella typhi (MTCC 733) .

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