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SYNTHESIS AND *IN VITRO* ANTIMICROBIAL STUDIES OF [1-(2, 4-DIHYDROXYPHENYL) ETHANONE-β-PHENYL ETHYL AMINE] AND ITS COMPLEXES

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ABSTRACT

The ligand [1-(2, 4-dihydroxyphenyl)ethanone-β-phenyl ethyl amine] was prepared by the reaction of [1-(2, 4-dihydroxyphenyl)ethanone with β-phenyl ethyl amine under reflux in ethanol. The complexes of this ligand have been prepared using metal acetates of Mn(II), Co(II), Ni(II), Cr(III), Cu(II), Zn(II) and Cd(II) under reflux in ethanol-DMF. The products were found to be crystalline solid. The ligand is characterized by analytical, FT-IR, proton NMR spectral data while complexes have been characterized by analytical, FT-IR and magnetic susceptibility measurements. The compounds were screened for antibacterial activity against some clinically important bacteria, such as *E. coli*, *S. typhy*, *S. aureus*, *P. aeruginosa* and *K. pneumonie* by using nutrient agar medium and antifungal activity against *C. albicans* and *A. niger* species by using potato dextrose agar medium.

Key words: β-Phenyl ethyl amine, DHPEPEA, Antibacterial, Antifungal.

INTRODUCTION

Schiff bases of acetophenone and their coordination compounds are known to possess the biological activities and inhibit many enzymatic reactions in the cell. Owing to their biological activities such as antifungal, antibacterial, antimycobacterial, antitumor, anti-inflammatory, anti-HIV, leishmanicidal, trypanocidal, inhibitor of anthrax lethal factor, antidiabetic, antimalarial, and antipyretic, there has been an increasing interest towards the studies of the coordination compounds of the Schiff bases during the past few decades[1-4]. The unique ability of transition metal ions and their complexes to control the chemistry of environmental, industrial, and biological processes has increased the importance of clarifying their mechanistic behavior in simple and complex chemical processes[5-7]. Schiff bases of substituted acetophenone and their derivatives are extensively studied as typical

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N, O donor ligands. Keeping in view the above importance of the compounds, we thought it worthwhile to synthesize and characterize the Schiff base and its coordination compounds with Mn(II), Co(II), Ni(II), Cr(III), Cu(II), Zn(II) and Cd(II) ions and these compounds have been studied for their antimicrobial activities.

EXPERIMENTAL

Materials and methods

Manganese(II), cobalt(II), nickel(II), chromium(III), copper(II), zinc(II), and cadmium(II) acetate salts used were of Merck and BDH make. Organic solvents such as absolute ethanol, methanol, petroleum ether, dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were of AR grade. The antibacterial activities of the compounds were assessed by using nutrient agar medium and antifungal activity by using potato dextrose agar medium.

Preparation of Schiff base ligand [DHPEPEA]

To the solution of [1-(2, 4-Dihydroxy-phenyl)-ethanone] (25 mL, 3.04 g, 0.02 mmole) in ethanol, β-phenyl ethyl amine (2.5 mL, 0.02 mmol) was added drop wise and the reaction mixture was refluxed on a water bath for 4.5 hrs. After cooling a reddish brown colored crystalline solid was separated out. It was filtered and washed with ethanol, crystallized from DMF and dried under reduced pressure at ambient temperature. The purity of ligand was checked by elemental analysis and m.p. It was also characterized by IR and ^{1}H NMR spectral studies. The purity of the synthesized compounds was monitored by TLC using silica gel (Yield = 79.5%).

Preparation of metal complexes

To a hot solution of ligand [DHPEPEA] (0.02 M) in 25 mL of ethanol-DMF with a solution of respective metal salts [acetates of Mn(II), Co(II), Ni(II), Cr(III), Cu(II), Zn(II) and Cd(II)] in ethanol was added drop wise with constant stirring. The reaction mixture was refluxed on a water bath for 3-5 hrs. The precipitated complexes were filtered, washed with ethanol followed by ether and dried over fused calcium chloride.

RESULTS AND DISCUSSION

The stoichiometry of the ligand and its complexes were confirmed by their elemental analysis. The elemental analysis of the ligand and its metal complexes show good support with the proposed structures of the ligand and its complexes and have been reported in Table 1.

Table 1: Analytical data of DHPEPEA and its complexes

S. No.	Compounds	Color	Time of reflux (hrs.)	Elemental analyses % Found (Calcd.)			
				M	C	H	N
1.	DHPEPEA	Dark red	4.5	--	75.02 (75.27)	6.22 (6.71)	5.78 (5.49)
2.	Mn-DHPEPEA	Golden brown	3.5	8.50 (8.64)	60.98 (60.47)	6.28 (6.34)	4.50 (4.41)
3.	Co-DHPEPEA	brown	3	9.87 (9.76)	63.73 (63.68)	6.09 (6.01)	4.70 (4.64)
4.	Ni-DHPEPEA	Reddish brown	4	9.82 (9.73)	63.74 (63.70)	5.99 (6.01)	4.58 (4.64)
5.	Cr-DHPEPEA	Golden buff	3	8.54 (8.46)	62.48 (62.53)	6.18 (6.23)	4.60 (4.56)
6.	Cu-DHPEPEA	Copper leaf	3.5	10.39 (10.45)	63.29 (63.20)	6.02 (5.97)	4.56 (4.61)
7.	Zn-DHPEPEA	Red buff	3	11.46 (11.39)	66.80 (66.96)	5.71 (5.62)	4.93 (4.88)
8.	Cd-DHPEPEA	Red buff	3	18.23 (18.10)	61.74 (61.89)	5.27 (5.19)	4.62 (4.51)

Spectral study

^{1}H NMR (300 MHz, $CDCl_3$, δ in ppm)[8-9]

7.46-6.75 (7H, m, Aromatic -OH), 2.1 (3H, s, $-CH_3$), 2.68-2.54 (2H, d, $-CH_2$), 3.76-3.65 (1H, t, -CH), 5.2-6.4 (1H, s, Aromatic –OH).

IR (KBr, cm^{-1})

In order to get bonding mode of ligand to metal in the complex, IR spectrum of the free ligand was compared with the spectra of metal complexes[10]. The IR spectrum of the Schiff base showed a band at about 1625 cm^{-1} due to (>C=N) azomethine group. The complexes displayed lower frequency shift of about ~15–20 cm^{-1}, suggesting coordination through the azomethine nitrogen. The spectrum of ligand shows a broad band at 3237cm^{-1} is assignable to intramolecular hydrogen bonded –OH stretch. New bands of weak intensity at 490-457 and 578-510 cm^{-1} in the metal complexes were tentatively assigned to the υ (M–O) and υ (M–N) modes, respectively. These new bands enhance the antimicrobial activity of complexes compared to Schiff base ligand[11]. The structurally important vibration bands of

the free ligands and their metal complexes, which are useful for determining the mode of coordination of the ligand are given in Table 2.

Table 2: IR spectral bands of DHPEPEA ligand and its complexes (in cm^{-1})

S. No.	Compounds	*ν*(O-H)/ *ν*(OH-N)	*ν*(C=N)	*ν*(C-O)	*ν*(M-N)	*ν*(M-O)	H_2O
1.	DHPEPEA	2925	1625	1290	--	--	3351, 1513, 827
2.	$[Mn(DHPEPEA)(H_2O)_2]2H_2O$	--	1595	1375	510	460	3212, 1532, 827
3.	$[Co(DHPEPEA)\ (H_2O)_2]$	--	1603	1387	514	490	3222, 1512, 840
4.	$[Ni(DHPEPEA)\ (H_2O)_2]$	--	1591	1360	525	470	3329, 1538, 820
5.	$[Cr(DHPEPEA)(H_2O)_2]H_2O$	--	1596	1367	520	457	3300, 1511, 834
6.	$[Cu\ (DHPEPEA)]2H_2O$	--	1580	1350	526	485	3439
7.	[Zn(DHPEPEA)]	--	1592	1325	521	488	---
8.	[Cd(DHPEPEA)]	--	1590	1320	578	470	---

Antimicrobial activity

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that inhibits the visible growth of a microorganism after overnight incubation. MIC of the various compounds against bacterial and yeast strains was tested through a modified agar well diffusion method[12]. In this method, a two-fold serial dilution of each compound was prepared by first reconstituting the compound in DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 512 to 1 µg/mL. 100 µL of each dilution was introduced into wells (in triplicate) in the agar plates already seeded with 100 µL of standardized inoculums (10^6 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37°C for 24 h, and the inhibition zones were observed. MIC was recorded for each test organism.

Antibacterial activity

To access the antibacterial activity of obtained compound Agar Well Diffusion method[13] was used. This activity was determined by using Mullar Hinton Agar[14]. A loop full

culture of each test organism were inoculated in sterilized nutrient agar and incubated overnight to obtain the broth culture. All the culture were inoculated on Mullar Hinton Agar plate by using sterile cotton swab after swabbing well was punched on media and the different dilutions of the compounds were added in to the well with the help of dropper. After addition of sample the plate were incubated at 37^{o}C for 24 hours. After incubation period plates were examined and zone of inhibition were measured.

Antifungal activity

The *in vitro* antifungal assay was performed by the disc diffusion method[15,16]. The complexes and ligand were tested against the fungi *Aspergillus niger*, and *Candida albicans*, cultured on potato dextrose agar as the medium. In a typical procedure, a well was created on the agar medium and nystatin as the control was inoculated with the fungi. The well was filled with the test solution, which diffuses and the growth of the inoculated fungi is affected. The inhibition zone, which developed on the plate was measured. The antimicrobial activity of ligand DHPEPEA and its complexes are shown in Table 3.

Table 3: Antimicrobial activity of ligand DHPEPEA and its complexes (diameter in mm)

Compound	Antibacterial					Antifungal	
	E. coli	***S. typhi***	***S. aureus***	***P. auruginosa***	***K. pneumonie***	***A. Niger***	***C. albicans***
DHPEPEA	13	15	12	14	14	18	14
Mn-DHPEPEA	14	--	13	18	19	20	23
Co-DHPEPEA	-	19	14	15	20	19	18
Ni- DHPEPEA	-	-	-	08	12	20	19
Cr- DHPEPEA	21	10	-	20	21	19	22
Cu-DHPEPEA	13	12	10	12	17	22	20
Zn- DHPEPEA	22	17	17	18	20	18	22
Cd-DHPEPEA	23	20	18	22	21	20	23
Amikacin	19	24	23	24	22	--	--
Fluconazole	-	-	-	-	-	26	24

Bacterial (zone of inhibition in mm) at 50 µg/mL, Fungal (zone of inhibition in mm) at 500 µg/mL.
Note: Including the well diameter of 6 mm. Zone of inhibition in mm (15 or less) resistant, (16-20 mm) moderate and (more than 20 mm) strongly active/sensitive

From the Table 3, it is clear that the Mn(II) complex shows strong activity against *A. niger*. Co(II) complex shows good antimicrobial activity against *S. typhi*, *K. pneumonia* and *A. niger*. Ni(II) complex shows good antimicrobial activity against *A. niger*. Cr(III) and Cu(II) complexes shows strong activity against *A. niger* and and *C. albicans,* while the complexes of Zn(II) and Cd(II) shows strong activity against nearly all microorganism indicates their good antimicrobial growth nature.

CONCLUSION

On the basis of the analytical data and other spectral techniques, it can be concluded that the complexes of DHPEPEA coordinating through its azomethine N and both enolic O atoms. The coordination compounds show significant enhanced antimicrobial activities as compared to the free Schiff base. Therefore, these compounds can be further used in pharmaceutical industry as antimicrobial agents for mankind, after testing its toxicity to human beings.

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