Synthesis, Characterization and Biological Studies of 2-(4-Nitrophenylaminocarbonyl)Benzoic Acid and Its Complexes with Cr(III), Co(II), Ni(II), Cu(II) and Zn(II)

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ABSTRACT: *Cr(III), Co(II), Ni(II), Cu(II) and Zn(II) salts of 2-(4-nitrophenylaminocarbonyl)benzoic acid were characterized by physical, analytical and spectroscopic studies and checked for their in-vitro antimicrobial activity against three bacterial strains, Mycobacterium smegmatis (Gram +ve), Escherichia coli (Gram -ve), Pseudomonas aeuroginosa (Gram -ve) and three fungal strains, Nigrospora oryzae, Aspergillus niger and Candida albicans. The antimicrobial activities of the metal complexes were found to be greater than those of 2-(4-nitrophenylaminocarbonyl)benzoic acid alone.*

KEY WORDS: *4-nitroaniline, Phthalic anhydride, Metal ions, Antimicrobial activity.*

INTRODUCTION

Resistance to the presently accessible antibiotics has motivated the search for new agents to inhibit bacterial activity. Among such agents, metal complexes of biologically active ligands are attractive as both ligands and metal ions can interact with different steps of pathogenic life cycles[1-3]. Much work has been done by bioinorganic as well as medicinal chemists to establish the relationship between the metal ions and their complexes as antitumour and antibacterial agents [4-8]. It is however remarkable that some biologically active compounds may become more carcinostatic and bacteriostatic upon chelation [9].

Aniline derivatives have been actively investigated because of their fascinating biological and diverse ligational behavior towards metal ions and novel structural features in their complexes [10-18] [19-21]. We have made studied the coordination and biological chemistry of 2-(4-nitrophenylaminocarbonyl)benzoic acid.

MATERIALS AND METHODS

All chemicals used were of analytical grade and were obtained from E-Merck/ BDH / Fluka. I.R spectra were recorded on a Philips analytical PU 9800 FTIR spectrophotometer (USA). U.V- visible spectra were obtained in DMSO by SPECORD-200 spectrophotometer (Analytik Jena, Germany) using ACTUA 710 software. Melting points were determined by a digital Gallen Kamp apparatus (UK).

Synthesis of Ligand

2-(4-nitrophenylaminocarbonyl)benzoic acid was made as described previously[15, 31].

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Table 1: Physical Properties of Ligand and its Metal Complexes.

S.No	Compound	Color	Physical State	Melting Point (°C)	μ_{eff}(B.M)	Conductance μS/cm	Yield %	λmax (cm^{-1})
1	Ligand	Yellowish brown	Crystalline	230	-	-	75	-
2	Cr(III)-complex	Dark Green	Amorphous	>280	3.81	121	68	15898, 23770
3	Ni(II)-complex	Brownish Yellow	Amorphous	>290	3.12	19	70	6312, 15433, 9789
4	Co(II)-complex	Light Pink	Amorphous	>291	4.76	33	56	7373, 17213, 20613
5	Cu(II)-complex	Green	Amorphous	>285	1.79	12	62	31055,19820
6	Zn(II)-complex	White	Amorphous	>267	Diamagnetic	14	43	27123

Ligand = 2-(4-nitrophenylaminocarbonyl)benzoic acid

Synthesis of Metal Complexes

Synthesis of Cu (II) – Com

2-(4-nitrophenylaminocarbonyl) benzoic acid (0.01 mol, 0.286 g) was suspended in acetone (100 mL) in a 250 mL two necked round bottom flask and then treated with triethylamine (0.45 mL). The mixture was refluxed for 2-3 h. Then $CuCl_2.2H_2O$ (0.005 mol) was added to the reaction flask with constant stirring and the reaction mixture was again refluxed for 8–10 h. A dark green precipitate was formed. The solvent was evaporated and the remaining mass was recrystallized from $CHCl_3$/ n-hexane mixture (1:1) and was stored in a moisture-free environment.

The Ni(II), Cr(II), Co(II) and Zn(II) complexes were made similarly from the hydrated chlorides.

RESULTS AND DISCUSSION

Physical analysis

2-(4-Nitrophenylaminocarbonyl)benzoic acid reacted with solutions of metal ions to give colored amorphous complexes of general formulae ML_2 and $ML_2(H_2O)_2$ as shown by elemental analysis (Table 3).

They were insoluble in common organic solvents and soluble in DMF and DMSO[19].

Electronic spectral analysis

Data obtained from the electronic spectroscopy are tabulated in Table 1. For the chromium complex two peaks at 15898 and 23770 cm^{-1} were assigned to $^4A_{2g} \rightarrow {}^4T_{2g}$ and $^4A_{2g} \rightarrow {}^4T_{1g}$ (f) d-d transitions, respectively. The appearance of these two bands confirms octahedral (O_h) geometry for this complex. The electronic spectrum of the Cu(II)- complex showed bands at 31055 cm^{-1} and 19820 cm^{-1} and are assigned, respectively, to charge transfer and $Eg \leftarrow {}^2T_{2g}$ transitions. These two band are in agreement with those usually observed for square planar Cu(II)-complexes [22]. The electronic spectrum of the Co(II) complex showed bands at 7373 cm^{-1}, 17213 cm^{-1} and 20613 cm^{-1} assigned to $^4T_{1g}$ $(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}$ $(F) \rightarrow {}^4T_{2g}(P)$ transitions, respectively, corresponding to octahedral geometry. The Ni(II)-complex also exhibited three spin-allowed bands at 26312 cm^{-1}, 15433 cm^{-1}, and 9789 cm^{-1}, assigned, respectively, to $^3A_{2g}(F) \rightarrow {}^3T_{2g}$ (F), $^3A_{2g}$ $(F) \rightarrow {}^3T_{1g}(F)$, and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$ transitions, consistent with well defined octahedral geometry. The diamagnetic Zn(II)-complex did not show any d-d transition.

Magnetic moment

The magnetic moment value for the Cr(III)-complex was found to be 3.81 BM (Table 1) which suggests an octahedral geometry for this complex [23]. The magnetic moment for value Cu(II)-complex (1.79 BM) also favors its square planar geometry. The magnetic moment value (3.12 BM) showed two unpaired electron per Ni (II) ion and is indicative of octahedral environment for Ni(II). Similarly the magnetic moment of the Co (II)-complex (4.76 BM) is consistent with octahedral geometry [23]. The Zn(II) complex was found to be diamagnetic

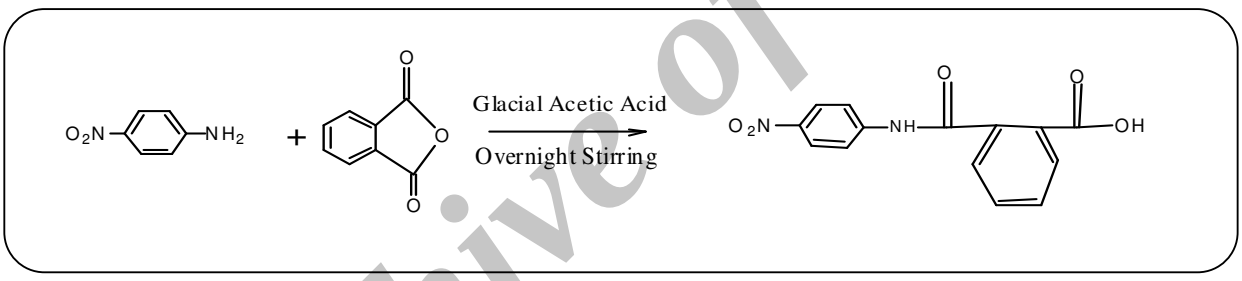
IR spectral analysis

The IR stretching frequencies and assignments are given in Table 2.

Table 2: IR stretching frequencies (cm^{-1}) of the various functional groups of ligand and its metal complexes

S.No	Compound	C=O cm^{-1}	NH cm^{-1}	(COO^-) asym cm^{-1}	(COO^-) Sym cm^{-1}	Δν cm^{-1}	(OH) cm^{-1}	C-H cm^{-1}	N-O asym. cm^{-1}	H_2O cm^{-1}	C-O-C cm^{-1}	C-N cm^{-1}
1	A	-	3479	-	-	-	-	794	1504	-	-	-
2	B	1762	-	-	-	-	-	798	-	-	1280	-
3	Ligand	1702	3379	1595	1377	218	2853	798	1518	-	-	1306
4	Cr(III)-complex	1730	3373	1553	1415	138	-	794	1525	3667	-	1295
5	Ni(II)-complex	1729	3378	1566	1381	185	-	709	1519	3643	-	1300
6	Co(II)-complex	1728	3376	1550	1378	172	-	783	1521	3455	-	1308
7	Cu(II)-complex	1729	3378	1552	1384	168	-	708	1514	-	-	1290
8	Zn(II)-complex	1732	3370	1559	1376	183	-	722	1513	-	-	1298

A= 4-Nitroaniline, B=Phthalic Anhydride = absent
Ligand = 2-(4-nitrophenylaminocarbonyl)benzoic acid



Scheme 1: Synthesis of 2-(4-nitrophenylaminocarbonyl)benzoic acid.

A carboxylate ligand can bind to the metal in monodentate or bidentate fashion, resulting in changes in the relative positions of the asymmetric and symmetric stretching vibrations [24]. The IR spectra of the complexes give a separation value (Δν) less than 200 cm^{-1}, which confirms that the coordination is bidentate [25-26]. A strong band at 3479-3437 cm^{-1}, characteristic of the NH group and present in the spectrum of the ligand precursor persists in the spectra of all metal complexes. This shows that NH group does not participate via intra or intermolecular interactions [27]. Peaks above 3400 cm^{-1} in the Co(II), Ni(II) and Cr(II) complexes, indicated the presence of coordinated water [13]. These were not present in the Cu(II) and Zn(II) complexes, suggesting that the coordination number in these compounds is only four. All the above discussion is consistent with the structures in Fig. 1.

Biological Studies

Antibacterial studies

Metal complexes were screened against three bacterial species like *Mycobacterium smegmatis, Escherichia coli, Pseudomonas aeuroginosa* by the paper disc diffusion method [13]. Studies of *in vitro* antibacterial activity of metal complexes exhibit appreciable antibacterial activity (Table 4) that is in accordance with our previous studies [28-29]. This enhanced antibacterial activity may be due to an increase in cell permeability of the lipophilic metal conjugates, which allows intracellular drug accumulation. It is also likely that the intracellular reduction of these metal compounds may lead to higher cytoplasmic concentration of metal species, which prove lethal for bacteria.

Table 3: Elemental analysis data of ligand and its metal complexes.

S.No	Compound	C % Obs. (Cal.)	H % Obs. (Cal.)	N % Obs. (Cal.)	M. % Obs. (Cal.)
1	Ligand	58.43 (58.95)	3.07 (3.16)	9.63 (9.82)	-
2	Cr(III)-complex	50.87 (51.09)	2.33 (2.73)	8.23 (8.51)	7.77 (7.90)
3	Ni(II)-complex	50.34 (50.57)	2.57 (2.71)	8.19 (8.42)	8.45(8.83)
4	Co(II)-complex	50.43 (50.55)	2.67 (2.70)	8.61(8.41)	8.47 (8.86)
5	Cu(II)-complex	52.87(53.06)	2.54 (2.84)	8.43 (8.84)	10.56 (11.14)
6	Zn(II)-complex	52.30 (52.90)	2.67 (2.83)	8.57(8.81)	11.10 (11.47)

Ligand = 2-(4-nitrophenylaminocarbonyl)benzoic acid

Table 4: Antibacterial activity of ligand and its metal complexes.

S.No	Compound	Bacterial Strain Inhibition Zone (mm)		
		A	B	C
1.	Sulphadimidine	+++	+++	++
2.	Ligand	+	-	+
3.	Cr(III)-complex	+ +	+	+
4.	Ni(II)-complex	+	+ +	-
5.	Co(II)-complex	+ +	+ +	+ +
6.	Cu(II)-complex	+ +	+ +	+ + +
7.	Zn(II)-complex	+ + +	+	+ +

Ligand = 2-(4-nitrophenylaminocarbonyl)benzoic acid.
A =E. coli, B =Pseudomonas aeuroginosa, C = Mycobacterium smegmatis.
Inhibition Zone = + 0-5 mm, ++ 6-0 mm, +++, 11-15mm, - inactive..

Table 5: Antifungal activity of ligand and its metal complexes.

S.No.	Compound	A			B			C		
		100µg/mL	500µg/mL	1000µg/mL	100µg/mL	500µg/mL	1000µg/mL	100µg/mL	500µg/mL	1000µg/Ml
1	Ketoconazole	10.5	15.8	27.9	15.5	23.8	31.9	12.3	17.7	33.9
2	Ligand	7.2	12.1	21.4	-	2.4	5.6	10.3	15.3	22.2
3	Cr(III)-complex	10.2	14.3	16.0	17.2	23.0	26.5	19.5	33.6	45.2
4	Ni(II)-complex	9.0	13.5	25.8	10.2	14.5	19.4	22.4	38.3	56.2
5	Co(II)-complex	15.2	33.5	54.4	16.2	30.3	44.5	13.3	24.5	34.4
6	Cu(II)-complex	22.6	47.5	63.4	17.3	27.7	54.2	21.5	35.2	49.4
7	Zn(II)-complex	21.2	35.5	53.4	15.2	28.3	39.5	13.3	29.5	39.4

Ligand = 2-(4-nitrophenylaminocarbonyl)benzoic acid
a. Nigrospora oryzae, b. Aspergillus niger, c. Candida albicans, - inactive.

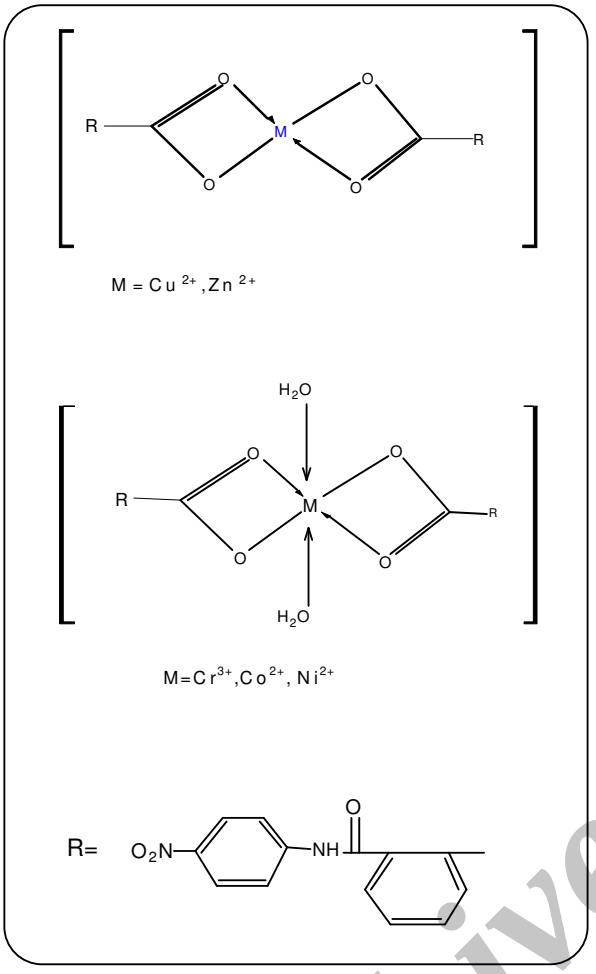


Fig. 2: Proposed structures of metal complexes.

Antifungal studies

The antifungal activity of all the synthesized metal complexes was determined against three fungal strains namely; *Nigrospora oryzae, Aspergillus niger* and *Candida albicans* by the agar plate technique and the results are recorded in Table 5. It was observed that metal complexes are more active than the corresponding ligand and toxicity increases with increasing concentration [30].

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