

Mixed Ligand Biologically Active complexes of Cobalt(II), Copper(II), Nickel(II) and Zinc(II) with Triazine-Derived NO and NS Donor Systems

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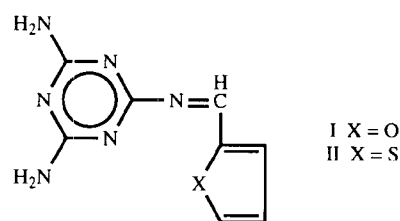
Summary: Biologically active triazine-derived NO and NS donor organic ligands and series of their cobalt(II), copper(II), nickel(II) and zinc(II) complexes have been synthesized. The ligands have been characterized by their IR, ¹H-NMR and elemental analysis whereas their metal complexes by conductance and magnetic measurements and elemental and spectral data. The synthesized ligands, in comparison to their metal complexes have also been screened for their antibacterial activity against bacterial species *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The activity data show the metal complexes to be more potent/antibacterial than the parent organic ligands against one or more bacterial species

Introduction

Many researches have drawn attention to the fact [1-6] that metals are essential for life. Certain diseases occur, only when an excess or deficiency of *in-vivo* metals appear. Paralleling these concepts and the significance of metals in biochemical processes, there ought to be an awareness of the usefulness of such researches. In this effort we have, commenced a research programme [7-9] to enlighten *in-vivo* role of metals (as being to lock the geometry of active sites so that only certain substrates can be accommodated, to activate enzymes or substrate bonds through coordination and to change the shape of the substrate so that it can fit into the active site) and focus upon metal chelates as being a wide range of new compounds worthy of consideration by therapeutic researches. Previously, we have studied bacteriostatic role of metals and have reported many biologically active compounds/drugs which via coordination with various metal ions become more bactericidal than the uncoordinated parent compounds [10-14]. In continuation to the same, we now wish to report newly synthesized biologically active compounds (I & II) which have been evaluated for their antibacterial properties in comparison to their metal complexes against bacterial species *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The *in-vivo* results of these studies show that the antibacterial activity of organic compounds has been substantially increased upon coordination/chelation.

Results and Discussion

The ligands (I & II) were prepared by adopting the same procedure as reported [15,16]



earlier. The structural determination of these ligands was done with the help of their IR, ¹H-NMR and microanalytical data (Table-1).

The tentative IR assignments of the free ligands show some characteristic bands at 3160, 1630, 1550 and 1615 cm⁻¹. These absorption bands are assigned to (-NH₂), azomethine linkage (C=N-), (C=C) and (C=N) heterocyclic, stretches respectively. The disappearance of band at 1760 cm⁻¹ and appearance of a new band at 1625 cm⁻¹ due to azomethine linkage confirm the formation of I and II. ¹H-NMR spectra (Table-1) also displays signals assigned to the expected number of protons. Also the microanalytical data CHN was found to be in complete agreement with molecular structures of the title ligands.

All the metal complexes [1-8] of these ligands were prepared by the stoichiometric reaction of the respective metal(II) chloride and ligands in molar ratio (M:L=1:2). All the complexes are air and moisture stable solids. They are soluble in DMF, DMSO and water and insoluble in other solvents. The conductivity of the complexes in aqueous solution (12-15 ohm⁻¹ cm² mol⁻¹) suggest their non-electrolytic nature [17,18].

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Table-1: Physical and elemental analysis and spectral data of ligands

Compound No.	Mol. formula	mp (°C)	IR(cm ⁻¹)	¹ H-NMR (ppm)	Analysis (%)		
					Calcd (Found)		
					C	H	N
I	C ₈ H ₆ N ₆ O	165	3160, 2930, 2020, 1695, 1630, 1615, 1605, 1550, 1115, 995, 880, 765	5.5 (4H, s, NH ₂), 6.8 (1H, dd, azomethine proton), 6.2 (2H, dd, H-1,2) 7.78 (1H, d, H-3)	47.05 (47.12)	3.92 (3.86)	41.17 (41.09)
II	C ₈ H ₆ N ₆ S	157	3160, 2935, 2810, 2675, 2020, 1690, 1630, 1615, 1605, 1550, 1110, 950, 885.	5.82 (4H, s, NH ₂), 6.8 (1H, d, azomethine proton), 6.21 (2H, dd, H-1,2) 7.8 (1H, d, H-3).	43.62 (43.88)	3.63 (3.41)	38.17 (38.22)

Magnetic moment

The magnetic susceptibility measurements (Table-2) for all the solid complexes are indicative of three unpaired electrons for Co(II) ion, one unpaired electron for Cu(II) ion and two unpaired electrons for each Ni(II) ion suggesting [19-21] octahedral geometry for Co(II) and Ni(II) complexes and distorted octahedral geometry for Cu(II) complexes. Their values towards slightly lower side are probably due to antiferromagnetism which occur via super exchange mechanism. The Zn(II) complexes were found to be diamagnetic.

Infrared spectra

The bonding of the ligands to the metal ion was investigated by comparing the IR spectra of the free ligand with metal complexes (Table-2) and the following conclusions were drawn.

a) The IR spectra of the ligands and their complexes exhibit a band at 3160 cm⁻¹ attributed to -NH₂ vibrations indicating [22] that the ligands are not attached to the metal atoms through these groups.

b) The IR spectra of complexes indicate that azomethine band in the spectra of ligands at 1630 cm⁻¹ is also shifted towards lower frequency by 5-10 cm⁻¹ respectively, indicating [23] the ligands to be coordinated to the metal ions through azomethine nitrogen.

c) The new bands appearing in the spectra of complexes and not observed in the spectra of ligands at 440-445 cm⁻¹ and 375-382 cm⁻¹ due to M-O and M-S modes [24] respectively indicate that ligands are also coordinated to the metal ions by the participation of oxygen and sulphur heteroatoms.

Table-2: Physical, spectral and analytical data of metal complexes

Complex No./ Mol. formula	mp(°C) (dec.)	BM (μ _B)	IR(cm ⁻¹)	λ _{max} (cm ⁻¹)	Analysis (%)		
					Calcd.(Found)		
					C	H	N
1 [Co(L ₁) ₂ Cl ₂]	238-4	4.18	3160, 2955, 2670, 1945, 1610, 1625, 1595, 1130, 1090, 870, 440.	19450, 17500, 8550	35.69 (35.76)	2.97 (2.83)	31.22 (31.27)
2 [Cu(L ₁) ₂ Cl ₂]	246-8	1.48	3160, 2950, 2675, 1940, 1600, 1620, 1595, 1135, 1095, 870, 445.	30675, 22220	35.39 (35.46)	2.94 (2.88)	30.97 (30.94)
3 [Ni(L ₁) ₂ Cl ₂]	255-7	3.25	3160, 2950, 2670, 1945, 1610, 1620, 1595, 1135, 1092, 870, 440.	25000, 15875, 9450.	35.71 (35.80)	2.97 (2.91)	31.25 (31.28)
4 [Zn(L ₁) ₂ Cl ₂]	215-8	Dia	3160, 2955, 2675, 1940, 1600, 1625, 1590, 1130, 1090, 870, 725, 445.	27550, 13220	35.27 (35.31)	2.93 (2.98)	30.86 (30.82)
5 [Co(L ₂) ₂ Cl ₂]	218-2	4.12	3160, 2955, 2875, 2670, 1945, 1615, 1625, 1595, 1130, 1090, 875, 775, 615, 375.	20200, 16250, 7500.	33.68 (33.66)	2.80 (2.89)	29.47 (29.46)
6 [Cu(L ₂) ₂ Cl ₂]	222-5	1.55	3160, 2955, 2870, 2675, 1945, 1610, 1625, 1595, 1135, 1095, 870, 770, 380.	29450, 23570.	33.41 (33.48)	2.78 (2.81)	29.23 (29.25)
7 [Ni(L ₂) ₂ Cl ₂]	236-8	3.32	3160, 2955, 2875, 2670, 1945, 1615, 1620, 1590, 1130, 1095, 775, 385.	26550, 14850, 10610	33.70 (33.78)	2.80 (2.86)	29.48 (29.55)
8 [Zn(L ₂) ₂ Cl ₂]	210-2	Dia	3160, 2950, 2875, 2670, 1945, 1615, 1620, 1595, 1130, 1095, 870, 775, 615, 380.	27500, 13220.	33.31 (33.37)	2.77 (2.71)	29.14 (29.06)

From the above observations, it is thus concluded that ligands behave as bidentate and possibly coordinate to the metal atoms through C=N and O or S groups by coordinate covalent bonding.

Electronic spectra

The electronic spectra of metal(II) complexes in water (Table 2) show different values with the changed metal ions. These are due to vibrations in perturbing influence of the metal ion on the ligand.

Cobalt(II) complexes exhibit absorption bands at 19450 and 20200, 16250 and 17500 and 7500 and 8550 cm^{-1} assigned to the transitions ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ (V_3), ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ (V_2) and ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (V_1) in octahedral geometry [25]. The spectra of Cu(II) complexes exhibit three broad bands in the region 29450 and 30675, 22220 and 23570 and 16250 and 17655 cm^{-1} . The lower energy band may be assigned to the transition ${}^2E_g \rightarrow {}^2T_{2g}$ as 10 Dq for distorted octahedral geometry [26]. The bands in the region 22220 and 23570 cm^{-1} can be attributed to ligand metal charge transfer.

The electronic spectra of Ni(II) complexes show d-d transitions in the region 25000 and 26550, 14855 and 15870 and 9450 and 10610 cm^{-1} . These are assigned to the transition ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$ (V_3), ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (V_2) and ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (V_1) respectively consistent with an octahedral environment [27]. The absorption spectra of Zn(II) complexes similarly, show a charge transfer band at 27550 cm^{-1} and a band at 13220 cm^{-1} due to transition ${}^2E_g \rightarrow {}^2T_{2g}$ in distorted octahedral environment [28].

Based on the knowledge gained from the above observations, it is proposed that all the complexes possess octahedral geometry in which the ligands behaving as bidentate arrange themselves around the metal atom and attain a stable configuration.

Antibacterial studies

The uncomplexed ligands and its metal complexes were tested for the antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antibacterial

Table-3: Antibacterial activity data of ligands and complexes

Ligand ^a /Complex No. ^b	Microbial species		
	a	b	c
I	++	++	+
II	++	++	++
1	+++	++++	++
2	+++	+++	++
3	+++	++++	+
4	++++	++++	++
5	++	+++	+++
6	+++	+++	++
7	++++	+++	+++
8	+++	++++	+++

a) Uncomplexed ligands (I,II), b) Same numbering as in Table-2. Microbial Species: A=*Escherichia coli*, b=*Pseudomonas aeruginosa*, C=*Staphylococcus aureus*. Inhibition zone measured in diameter, + 6-10 mm; ++ 10-14 mm; +++ 14-18 mm; +++++, > 20mm.

activity was tested at a concentration of 30 $\mu\text{g}/0.01$ ml in DMF using paper disc diffusion method as reported earlier [29,30]. The results of these studies reported in Table-3 showed that ligands and all their metal complexes are biologically active against one or more bacterial species and the metal complexes have been shown to be more antibacterial than the simple uncomplexed parent organic compounds.

Experimental

All chemicals and solvents used were AR grade. Metals were used as their chloride salts in the preparation of complexes. Infrared spectra were recorded on A-10 spectrophotometer in nujol. Elemental analysis of CHN was carried out on a Coleman automatic analyzer. Electronic spectra were recorded on a Hitachi double-beam U-2000 spectrophotometer using glass cells of 1 cm thickness. Conductance was measured on a conductance meter YSI model-32 and magnetic susceptibility on a Gouy's balance. All melting points were taken on a Gellenkamp melting point apparatus and are uncorrected.

Antibacterial activity of the ligands and their metal complexes was studied on wild type pathogenic bacterial species collected from different patients admitted in Bahawal Victoria Hospital, Bahawalpur. These studies were carried out with the help of Microbiology Laboratory, Department of Pathology Quaid-e-Azam Medical College, Bahawalpur.

Preparation of Ligands

(N-(2-Furanylmethylene)2,4,6-triamino-1,3,5-triazine(I)

Furan-2-carboxaldehyde (1.92 ml, 1.65 g, 0.01 mol) in ethanol (15 ml) was added to a hot ethanolic solution (20 ml) of melamine (1.26 g, 0.01 mol). The 2-3 drops of concentrated sulphuric acid were added and this mixture refluxed for 2h, during which a solid product was formed. The resultant mixture was then cooled, filtered, washed with hot ethanol (2 x 5 ml) and dried to give (I) (52%).

N-(2-Thienymethylene)2,4,6-triamino-1,3,5-triazine(II).

Thiophene-2-carboxaldehyde (0.92 ml, 1.12 g, 0.01 mol) in ethanol (15 ml) was added to a hot and magnetically stirred ethanolic solution (20 ml) of melamine (1.26 g, 0.01 mol). A few drops of concentrated sulphuric acid were then added and the mixture refluxed for 2h. The solution on cooling gave a light yellow product which was filtered, washed with a little hot ethanol (2 x 5 ml) and dried. The solid thus obtained was crystallized in aqueous ethanol to give (II) (50%).

Preparation of metal complexes

A hot solution of ligand (0.002 mol) in absolute ethanol (25 ml) was added to a magnetically stirred aqueous solution (15 ml) of metal (II) chloride (0.001 mol). The mixture was refluxed for 1h. A solid product was formed during refluxing and the reaction mixture was cooled, washed with ethanol (2 x 10 ml) and dried. The product formed was recrystallised from aqueous ethanol to give 1 (48%), 2 (50%), 3 (43%), 4 (46%), 5 (45%) 6 (48%), 7 (45%) and 8(47%).

Antibacterial studies

Preparation of disc

A solution of the ligand/complex (30 µg) in DMF (0.01 ml) was applied to a disc prepared from blotting paper (3 mm size) with the help of a micropipett. The discs were left in an incubator for 48 hr at 37°C and then applied to a bacteria grown agar plates.

Preparation of agar plates

Agar plates were prepared according to the reported method described earlier by us [12-14]

using a specific agar medium for the growth of specific bacterial strains.

Procedure of inoculation

It was done by means of a platinum wire loop which was first made red hot on a flame, allowed to cool in air and then used for the application of previously described bacterial strains. The preculture was first prepared in 2 ml of nutrient broth by selecting a suitable bacterial colony and later on transferred to a nutrient broth which was incubated for 2h at 37°C. Then 500 µl of the culture was speared on the agar plate and incubated for 2h at 37°C.

Application of disc

A sterilized forceps was used for the application of paper discs to the already incubated agar plates. When the discs were applied, the plates were incubated at 37°C for 24 h.

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