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BIOLOGICAL ROLE OF ANIONS (SULFATE, NITRATE, OXALATE AND ACETATE) ON TIIE PHARMACOLOGICAL PROPERTIES OF COBALT (II) AND NICKEL(II) CHELATES WITH THIENOYL - AND FURENOYL - DERIVED COMPOUNDS

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ABSTRACT

Biologically active *complexes* of cobalt (II) and nichd (II) with thienoyl - and furenoyl-derived Schiff-base ligands. having Ore same metal ion but different anions such as sulfate, nitrate, oxalate and acetate have been prepared and characterized. In order to evaluate the role of anions on their pharmacological proper-tics the synthesized complexes have been screened against bacterial species. *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* and results have been reported.

INTRODUCTION

Sulfur, Oxygen and Nitrogen have long been used (Perrin, 1964) as heteroatoms to increase the biological activity of an organic moiety. *The in vivo* interaction of metal ions with such biological active compounds is of immense biological importance. There have been numerous reviews (Sigel and McCormick. 1970; Dixon and Wabb, 1964; Vallee and Coleman. 1964, Sander et al., 1965) which show that the functioning of many enzymes is metal ion dependent and therefore, emphasize on the biological significance (Sinn and Harris, 1969, Tsibris and Woody, 1970) of metal ions. Those factors, which potentiate metal ion binding to enhance the biological activity, are still to be determined. For this purpose we have commenced a research p*ro*gram (Chohan and Raul 1996: Cholnn and Sherazi, 1997; evaluating those possible factors which tend to make the metal ion binding/coordination more biologically active or bactericidal.

In continuation to the same we have prepared metal complexes of the type $IM(L)_2X]$ IM=Co(II) & Ni(II), L=HL^1 & HL^2 (Fig. 1) and X=SO_4^{2-}, NO_3, $C_2O_4.^{2-}$ & CH_3CO_2] having the same metal ion but different anion which stay as counter ions in the title complexes and their possible effect which may have on the role of metal ion is determined and reported in this paper.

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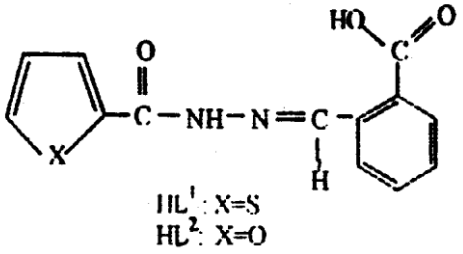

(Figure 1: Structure of Ligand)

EXPERIMENTAL

Material and Methods

All chemicals and solvents used were of Analar grade. All the metals were used as their metal (II) salts. IR spectra were recorded on Philips Analytical PU9800 FTIR UV-Visible spectra were obtained on a Hitachi U-2000 double-beans spectrophotometer Conductance of the metal complexes was determined in DMF at 10^{-3} dilution on a YSI 32 model conductor meter. Magnetic measurements were done on solid complexes using the Golly method. The synthesized metal chelates were analyzed for C, H and N by microanalytical techniques and the metal content in the chelates were estimated by the standard methods. Melting points were recorded on a Gallenkamp apparatus and are uncorrected. The antibacterial studies were carried out with the help of the Department of Pathology, Quaid-e-Azam Medical College, Bahawalpur, Pakistan.

Preparation of Ligand

Ligands were prepared by the method reported (Chohan et al. in press) earlier by us.

Preparation of Metal Complexes

An ethanol solution of appropriate metal(II) salt (1 mmol. ml) was added to a stirred hot ethanol solution of the respective ligand (2 mmol. 30 ml). The resulting mixture was reflaxed for 2 h. The solution was then cooled, filtered and reduced to nearly half volume. Adding ether in excess and immediately precipitating effected the metal complexes. The precipitates were filtered, washed with ethanol, then with ether and dried. These were crystallized for hot aqueous ethanol to give metal complexes (1-16).

ANTIBACTERIAL STUDIES

Preparation of Discs

The ligand/complex (30 mg) in DMF (0.01 ml) was applied on a paper disc, [prepared from blotting paper (3 mm diameter)] with the help of a micropipette. The

discs, were left in an incubator for 48 h at 37°C and then applied on the bacteria grown agar plates.

Preparation of Agar Plates

Minimal agar was used for the growth of specific bacterial species. For the preparation of agar plates for *Escherichia coli*, MacConkey agar (50 g), obtained from Merck Chemical Company, was suspended in freshly distilled water (IL). It was allowed to soak for 15 minutes and then boiled on a water bath until the agar was completely dissolved the mixture was autoclaved for 15 minutes at 120°C and then pouted into previously washed and sterilized petri dishes and stored at 40°C for inoculation.

Procedure of Inoculation

Inoculation was done with the help of a platinum wire loop which was made red hot in a fame, cooled and then used for the application of bacterial strains.

Application of Discs

A sterilized forceps was used for the application of paper disc on the already inoculated agar plates. When the discs were applied, they were incubated at 37° C for 24 h. The zone of inhibition was then measured (in diameter) around the disc.

RESULTS AND DISCUSSIONS

The reaction of ligands with metal(II) chloride salts yielded complexes of 1:2 (M:L) stoichiometric composition. All complexes are air/moisture stable solids. They are insoluble in common organic solvents such as chloroform, ethanol, acetone, dichloromethane and benzene, partially soluble in water and completely soluble in DMF and DMSO the conductivity measurements (15-28 ohm^{-1} cm^2 mol^{-2}) of these complexes in DMF indicated (Shallary et al, 1979; Geary, 1971) them to be non-electorlytes.

Infrared Spectra

The important infrared spectral band of the metal chelates are discussed in Table I. The band due to the azomethine a (C=N) linkage appeared at 1635 cm^{-1} in the case of all free ligands. A lower frequency shift of this band (~15-20 cm^{-1}) observed in the case of its metal chelates, indicated coordination through this azomothine nitrogen. The amide υ (NH) band appearing at 3220 cm^{-1} in the free ligand remains unaffected in the its metal complexes, which clearly indicated that the inane nitrogen is not taking part in the coordination. The band appearing at 1735 cm^{-1} suffered a negative (~25 cm^{-1}) shift indicating coordination through the carbonyl oxygen atom of carbonyl group. The carboxylic υ (COOH) group stretching vibrations were found at ~1680 cm^{-1} in the free ligands which also shifted to lower frequency (~8-14 cm^{-1}) in the case of the metal complexes, showing coordination through this catboxvlic group by deprotonation. The coordination of the met-al through the ammethine nitrogen and oxygen donor sites of the ligand was further con-firmed by the appearance of the new bands in the regions 405-450 cm^{-1} and 455-510cm^{-1} which could be assigned (Nakamoto, 1978) to their respective M-N and M-O frequencies.

Table 1
Physical, Spectral and Analytical Data of The Metal Complexes

Metal Complex/ Mol. Formula	M.P. (°C)	B.M. (μ_{eff})	IR (cm^{-1})	λ_{max} (cm^{-1})	Calc (Found) % C	H	N
1 $[Co(HL^1)_2(SO_4)_2]$ $C_{26}H_{14}CoN_4O_{14}S_4$ [793.4]	292-294	3.78	3220, 1710, 1675 1615, 515, 405	9045, 15215, 27210	39.4 (40.1)	1.8 (1.9)	7.1 (7.2)
2 $[Co(HL^1)_2(NO_3)]$ $C_{26}H_{14}CoN_5O_9S_2$ [663.1]	286-288	4.11	3220, 1720, 1675, 1620, 515, 410	8555, 15348 26608	47.1 (47.3)	2.1 (2.5)	10.6 (10.4)
3 $[Co(HL^1)_2(C_2O_4)_2]$ $C_{30}H_{14}CoN_4O_{14}S_2$ [777.4]	277-279	3.88	3220, 1725, 1672, 1625, 515, 405	10515, 17535, 28375	46.3 (46.8)	1.8 (1.8)	7.2 (7.1)
4 $[Co(HL^1)_2(CH_3CO_2)]$ $C_{28}H_{17}CoN_4O_8S_2$ [660.3]	285-287	3.91	3220, 1720, 1670, 1625, 515, 410	9915, 16875 28955	50.9 (51.2)	2.6 (2.3)	8.5 (8.8)
5 $[Co(HL^2)_2(SO_4)_2]$ $C_{26}H_{14}CoN_4O_{16}S_2$ [761.3]	276-278	4.0	3220, 1725, 1670, 1625, 515, 410	9918, 16860 28950	41.0 (4.1)	1.8 (1.5)	7.4 (7.3)
6 $[Co(HL^2)_2(NO_3)]$ $C_{26}H_{14}CoN_5O_{11}$ [631.2]	268-270	3.97	3220, 1720, 1675 1620, 515, 410	8550, 15347, 26600	49.5 (49.3)	2.2 (2.5)	11.1 (10.8)
7 $[Co(HL^2)_2(C_2O_4)_2]$ $C_{30}H_{14}CoN_4O_{16}$ [745.2]	279-281	4.10	3220, 1725, 1672, 1622, 515, 410	10512, 17530, 28377	48.3 (48.8)	1.9 (1.8)	7.5 (7.5)

Metal Complex/ Mol. Formula	M.P. (°C)	B.M. (μ_{eff})	IR (cm^{-1})	λ_{max} (cm^{-1})	Calc (Found) % C	H	N
8 $[Co(HL^2)_2(CH_3CO_2)]$ $C_{28}H_{17}CoN_4O_{10}$ [628.2]	265-267	3.90	3220, 1720, 1670, 1625, 515, 410	9915, 16865, 28955	53.5 (53.2)	2.7 (2.3)	8.9 (8.8)
9 $[Ni(HL^1)_2(SO_4)_2]$ $C_{26}H_{14}NiN_4O_{14}S_4$ [793.2]	288-290	3.10	3220, 1715, 1670, 1620, 510, 405	9045, 15215, 27215	39.4 (40.1)	1.8 (1.9)	7.0 (7.2)
10 $[Ni(HL^1)_2(NO_3)]$ $C_{26}H_{14}CoN_5O_9S_2$ [663.1]	291-293	2.72	3220, 1720, 1675, 1625, 515, 415	8555, 15345, 26600	47.1 (47.3)	2.1 (2.4)	10.6 (10.5)
11 $[Ni(HL^1)_2(C_2O_4)_2]$ $C_{30}H_{14}NiN_4O_{14}S_2$ [777.1]	279-281	2.78	3220, 1725, 1672, 1622, 515, 405	10512, 17535, 28375	46.4 (46.5)	1.8 (1.8)	7.2 (7.6)
12 $[Ni(HL^1)_2(CH_3CO_2)]$ $C_{28}H_{17}NiN_4O_8S_2$ [660.1]	275-277	2.81	3220, 1720, 1675, 1625, 515, 410	9915, 16865, 28950	50.9 (50.7)	2.6 (2.4)	8.5 (8.8)
13 $[Ni(HL^2)_2(SO_4)_2]$ $C_{26}H_{14}NiN_4O_{16}S_2$ [761.1]	271-273	2.95	3220, 1725, 1675 1625, 510, 410	9918, 16865, 28950	41.0 (41.1)	1.8 (1.5)	7.4 (7.3)
14 $[Ni(HL^1)_2(NO_3)]$ $C_{26}H_{14}NiN_5O_9S_2$ [631.0]	277.279	2.86	3220, 1725, 1675, 1620, 515, 410	8550, 15345, 26605	49.5 (49.3)	2.2 (2.2)	11.1 (10.4)
15 $[Ni(HL^1)_2(C_2O_4)_2]$ $C_{30}H_{14}NiN_4O_{14}S_2$ [745.0]	268-270	3.08	3220, 1725, 1672, 1622, 515, 405	10515, 17533, 28375	48.4 (48.5)	1.9 (1.8)	7.5 (7.3)
16 $[Ni(HL^1)_2(CH_3CO_2)]$ $C_{28}H_{17}NiN_4O_8S_2$ [628.0]	279-281	2.89	3220, 1720, 1670 1625, 515, 405	9920, 16868, 28955	53.6 (53.8)	2.7 (2.9)	8.9 (8.8)

UV-Visible Spectra and Magnetic Moments

The magnetic moment and electronic spectral data of the cobalt(II) and nickel(II) complexes were calculated from the corrected magnetic susceptibility values and suggested (Carlin, 1978) an octahedral geometry for all the complexes around the metal ion. The room temperature magnetic susceptibility (Table 1) for the solid complexes lie within the range excepted for their octahedral geometry. Three unpaired electrons per Co(II) ion (μ_{eff} = 3.85 - 4.27 B.M.) and two unpaired electrons per Ni(II) ion (μ_{eff} = 2.85 - 304 B.M.) suggested (Balhausert, 1962; Glick and Lintvedt. 1976) octahedral geometry for Co(II) and Ni(II) complexes. The magnetic susceptibilities have, in general, lower values than the expected moments probably due to antiferrotmxgnetism, which arises via a super-exchange mechanism.

The UV-Visible spectra of the cobalt(II) chelates showed three bands observed at 8555-9045 cm^{-1}, 15345-15215 cm^{-1} and 26600-27210 cm^{-1} in its electronic spectra which may be assigned to $4T^{lg} \rightarrow T_{2g}$ (F), $^4T_{1g} \rightarrow 4^A{}_{2g}$ (F) and $^4T_{1g} \rightarrow {}^4T_{lg}$ (P) transitions, respectively, and are suggestive (Lever, 1984; Meek et al., 1962) of its idealized octahedral geometry around the cobalt ion. The nickcl(II) chelates showed also three bands observed at 9918-10512 cm^{-1}, 16865-17531 cm^{-1} and 28375-28950 cm^{-1} due to spin-allowed transitions from $^3A_{2g} \rightarrow {}^3$T2g (F), $^3A_{2g} \rightarrow {}^3A_{1g}$ (F) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P), respectively. in an octahedral environment (Figgis and Lewis, 1964).

In light of the above observations it is suggested that all the metal chelates show an octahedral geometry in which the two ligand molecules act as tridentate around the metal atom, accommodating themselves in such a way that a stable chelate ring is formed.

Antibacterial Studies:

The title ligands in comparison to their metal complexes, were screened against the bacterial species *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* in order to determine the effect of metal ions on the individual antibacterial activity of metal chelates.

Table 2
Antibacterial Activity Data of Metal Chelates

Ligand/Chelate	Microbial Species		
	a	b	c
HL^1	++	+	+
HL^2	+	++	-
1	++++	++	++
2	+++	+++	+++
3	+++	+++	+++
4	+++	+++	+++
5	+++	+++	++
6	+++	+++	++
7	+++	++	+++
8	++++	+++	++++
9	+++	+++	++
10	+++	++++	+++
11	+++	+++	+++
12												
13	++++	++	+++
14	++	++++	++
15	+++	+++	+++
16	+++	++	++++

a = *Escherichia coli*, b = *Pseudomonas aeruginosa*, c = *Staphylococcus aureus*
Inhibition zone diameter mm (% inhibition): +, 6-10 (27-45%); ++, 10-14 (45-64%); 14-18 (54-82%); , 18-22 (82-100%). Percent inhibition values are relative to inhibition zone (22 mm) of the most active compound with 100% inhibition.

The compounds were tested at a concentration of 30 µg/0.01 ml in DMF solution using paper disc diffusion method. The results of these studies reproduced in Table 2 clearly indicated that when the same metal chelate having different anions was individually screened, the degree of bactericidal activity varied from anion to anion. For example, the Co(II) complex having nitrate anion was more bactericidal than the Co(II) complex with sulphate, oxalate or acetate anions. Similarly, the Co(II) complex of oxalate anion was more antibacterial active than the complex with acetate chloride or sulfate anions. The same results were found for NMI) complexes. From the obtained data, it was generally observed that the order of potency in comparison to the metal complexes having chloride anions evaluated and reported earlier (Chohan et al., in press) and to the results of the present studies for the anions sulfate nitrate, oxalate and acetate against the tested bacterial species was found to follow the order as:

$NO_3 > C_2O_4 > CH_3CO_2 > Cl > SO_4$

On the basis of these results, we strongly claim that different anions do effect the biological behaviour of the metal chelates. However, at this stage, we are not aware of the actual mechanism. It is however, suspected that factors such as stability constant solubility, conductivity, dipole moment and cell permeability mechanism (influenced by the presence of anions) may be the possible reasons for effecting the activity.

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