

## **ANTIBACTERIAL COBALT(II) AND ZINC(II) COMPLEXES OF PYRAZINE-DERIVED NNO AND NNN DONOR SCHIFF-BASES**

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### **ABSTRACT:**

A series of biologically active pyrazine-derived Schiff-base ligands have been synthesized by the condensation reaction of 2-aminopyrazine with salicylaldehyde and acetylacetonide. Then their cobalt(II), nickel(II) and zinc(II) complexes have been prepared. The biological evaluation of the simple uncomplexed ligand in comparison to their complexed compounds have been determined against bacterial strains e.g., *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

### **INTRODUCTION**

Schiff-bases and their metal complexes are important intermediates (Snell and Metzler, 1968; Holm et al, 1966; Weinstein et al., 1970) in certain biological processes. Much work has been reported (Bhaskare and More, 1986; Duta and Hossain, 1985; Clein et al., 1983; Fleischer and Lawson, 1972; Chohan, 1999; Chohan and Praveen, 1999; Chohan, 1999; Chohan and Kausar, 2000; Chohan and Praveen, 2000) on the Schiff-bases due to their interesting ligational as well as biological behavior. Despite the controversy that all viruses are cancer-producing agents but, there is no longer any question that certain types of cancers are virus caused, if not all, most of the cancers have one or more viruses associated with them (Luria, 1960). Since viruses contain proteins and nucleic acid units and some atoms of which are excellent coordinating agents, therefore, it attracts the attention of researchers to study their interesting ligational and biological behavior. Previously we have extensively studied (Chohan and Praveen, 1998; Chohan and Sherazi, 1999; Chohan and Pervez, 1993; Chohan et al, 1997; Chohan et al, 1998; Chohan et al, 1998; Chohan and Rauf, 1996; Chohan and Sherazi, 1997) the possibility of altering the biological activity via its coordination and/or chelation to the metal atoms with the expectation that this alteration will result in the elimination or diminution of its biological activity. In continuation to the same, we now wish to report novel biologically active Schiff-base compounds (Fig. 1) synthesized in our laboratory and their evaluation caused by the effect of chelation/coordination on their biological activity.

### **EXPERIMENTAL**

#### ***Material and Methods***

All chemicals and solvents used were of Analar grade. All metal(II) salts were used as chlorides. IR spectra were recorded on a Philips Analytical PU 9800 FTIR spectrophotometer as KBr discs. UV-Visible spectra were obtained in DMF on a Hitachi U-2000 double-beam spectrophotometer. C, H, N analyses was carried out by Butterworth Laboratories Ltd. Conductance of the metal complexes was determined in DMF on a Hitachi YSI-32 model conductometer. Magnetic measurements were made on solid complexes using the Gouy method. Melting points were recorded on a Gallenkamp apparatus and are uncorrected.

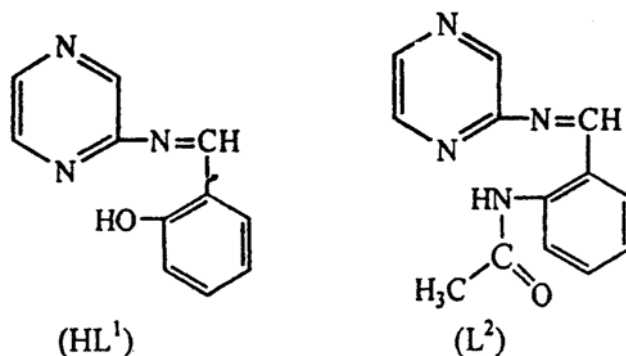


Fig. 1: Structure of the Schiff-base Ligands

#### **Preparation of Schiff-base Ligand (HL<sup>1</sup>)**

Salicylaldehyde (1.2 g, 1.1 mL, 0.01 M) in ethanol (10mL) was added to an ethanol solution (20 mL) of 2-aminopyrazine (0.95 g, 0.01 M). Then 2-3 drops of conc. H<sub>2</sub>SO<sub>2</sub> were added and mixture refluxed for 2 h. On cooling, a solid product was formed which was filtered, washed with ethanol, then with ether and dried. Crystallization in hot ethanol gave HL<sup>1</sup> (1.2 g, 60%), M.p 114°C. The same method was applied for the preparation of L<sup>2</sup> (1.6 g, 66%). M.p 125° C by using the respective reagents in the same molar ratio.

#### **Preparation of Cobalt(II) Complex of (HL<sup>1</sup>)**

A warm ethanol solution (20 mL) HL<sup>1</sup> (0.4 g, 0.002 M) was added to a magnetically stirred solution of cobalt(II) chloride hexahydrate (0.2 g, 0.001 M) in distilled water (25 mL). The mixture was refluxed for 1 h and cooled to room temperature. On cooling, pink precipitates were formed which were filtered, washed with ethanol, acetone and ether, and dried by suction. Crystallization in aqueous ethanol (70:30) gave the desired metal complex (1) (0.5 g, 68%). All other metal complexes were prepared respectively following the same method.

#### **Antibacterial Studies**

The synthesized metal complexes, in comparison to the uncomplexed Schiff-base ligands were screened for their antibacterial activity against pathogenic bacterial species, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The paper disc diffusion method (Chohan et al, 1999) was adopted for the determination of antibacterial activity.

## **RESULTS AND DISCUSSION**

#### **Physical Properties**

The Schiff-bases (HL<sup>1</sup> and L<sup>2</sup>) (Fig. 1) were prepared by refluxing an appropriate amount of 2-aminopyrazine and the respective aldehyde in hot ethanol in 1 : 1 molar ratio. The structures of these Schiff-base ligands were established with the help of their IR, NMR, and microanalytical data (Table 1).

All of the metal complexes (1-6) (Table 2) of these Schiff-bases were air stable and prepared by the stoichiometric reaction of the respective metals as their chlorides and the respective Schiff-bases in molar ratio (M:L<sup>-1</sup>:2). The complexes are intensely colored and amorphous solids, which decompose. They are insoluble in common organic solvents like ethanol, methanol, chloroform or acetone but only soluble in DMSO and DMF. Their solubility nature and elemental analysis data suggest that they are monomers. Molar conductance values of the soluble complexes in DMF show low value (11-18 ohm<sup>1</sup>cm<sup>2</sup>mol<sup>-1</sup>) indicating that they are all non-electrolytic in nature (Geary, 1971; Shallary et al, 1979).

### Infrared spectra

IR spectra of the Schiff-bases showed the absence of bands at 1735 and 3320 cm<sup>-1</sup> due to the carbonyl  $\nu(\text{C}=\text{O})$  and  $\nu(\text{NH}_2)$  stretching vibrations and instead, a strong new band appeared at ~1635 cm<sup>-1</sup> assigned to the azomethine  $\nu(\text{HC}=\text{N})$  linkage. It in turn, suggested (Bellamy, 1968) that amino and aldehyde moieties of the starting reagents no more existed and have been converted into their respective Schiff-base compounds (Fig. 1). The comparison of the infrared spectra of the Schiff-bases and their metal chelates indicated that the Schiff-bases were principally coordinated to the metal atom in three ways, thus representing the ligands to act as tridentate. The band appearing at 1630 and 1635 cm<sup>-1</sup> due to the azomethine shifted to lower frequency by ~10-15 cm<sup>-1</sup> indicating participation of the azomethine nitrogen in the complexation. The band at 1310 cm<sup>-1</sup> assigned to the pyrazine ring  $\nu(\text{C}=\text{N})$  nitrogen also shifted to lower frequency by ~10 cm<sup>-1</sup> which was also indicative of the involvement of the pyrazine ring nitrogen in the coordination. A broad band appearing at 3415 cm<sup>-1</sup> assigned to  $\nu(\text{OH})$  in the Schiff-base ligand (HL<sup>1</sup>) was no more found in the spectra of its metal complexes indicating deprotonation and coordination of the hydroxyl oxygen to the metal atom. The band appearing at 3275 cm<sup>-1</sup> assigned to  $\nu(\text{NH})$  in the ligand (L<sup>2</sup>) also shifted to the lower frequency by 10-15 cm<sup>-1</sup> in the spectra of its complexes indicating participation of NH group in the coordination. Further conclusive evidence of the coordination of these Schiff-base compounds with the metals, was shown by the appearance of weak low frequency new bands at 525-530 and 455-460 cm<sup>-1</sup> (Table 3). These were, assigned (Nakamoto, 1978) to the metal-nitrogen  $\nu(\text{M}-\text{N})$  and metal-oxygen  $\nu(\text{M}-\text{O})$ , respectively. These new bands were only observable in the spectra of the metal complexes and not in the spectra of the uncomplexed Schiff-base compounds, thus, confirming participation of the groups (O or N) in the coordination.

**Table 1**  
Physical, Spectral and Analytical Data of the Schiff-bases

Schiff-base	IR (cm <sup>-1</sup> )	<sup>1</sup> H NMR (DMSO-d <sub>6</sub> ) ppm	Calc (Found) %		
			C	H	N
HL <sup>1</sup> C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O [199.0]	3415 (br, OH), 1630 (s, HC=N), 1310 (s, Py).	9.9 (s, 1H, OH), 8.6 (s, 1H, HC=N), 7.8 (m, 2H, Py), 7.6 (m, 1H, Py), 7.4 (m, 1H, Ph), 7.2 (m, 1H, Ph), 7.1 (m, 1H, Ph), 6.9 (M, 1H, Ph).	66.3 (66.6)	4.5 (4.2)	21.1 (21.5)
L <sup>2</sup> C <sub>13</sub> H <sub>12</sub> N <sub>4</sub> O [250.0]	3275 (s, NH), 1635 (s, HC=N), 1315 (s, Py), 1735 (C=O).	8.8 (s, 1H, HC=N), 8.3 (s, 1H, NH), 7.9 (m, 2H, Py), 7.7 (m, 1H, Py), 7.6 (m, 1H, Ph), 7.4 (m, 1H, Ph), 7.2 (m, 1H, Ph), 7.1 (m, 1H, Ph), 2.8 (s, 3H, CH <sub>3</sub> ).	62.4 (62.8)	4.8 (4.5)	22.4 (22.2)

**Table 2**  
Physical and Analytical Data of the Metal(II) Complexes

S. No.	Metal chelate/Mol. Formula	Yield (%)	M.p(°C) (decomp)	B.M. ( $\mu_{\text{eff}}$ )	Cal (Found) %		
					C	H	N
1	[Co(L <sup>1</sup> ) <sub>2</sub> ] [454.9]	59	189-191	4.2	58.0	3.5	18.5
	C <sub>22</sub> H <sub>16</sub> CoN <sub>6</sub> O <sub>2</sub>				(58.4)	(3.7)	(18.6)
2	[Ni(L <sup>1</sup> ) <sub>2</sub> ] [454.7]	55	204-206	3.1	58.0	3.5	18.5
	C <sub>22</sub> H <sub>16</sub> NiN <sub>6</sub> O <sub>2</sub>				(58.2)	(3.9)	(18.2)
3	[Zn(L <sup>1</sup> ) <sub>2</sub> ] [461.4]	58	210-212	Dia	57.2	3.5	18.2
	C <sub>22</sub> H <sub>16</sub> ZnN <sub>6</sub> O <sub>2</sub>				(57.1)	(3.8)	(18.5)
4	[Co(L <sup>2</sup> ) <sub>2</sub> ]Cl <sub>2</sub> [609.8]	58	205-207	4.4	51.2	3.9	18.4
	C <sub>26</sub> H <sub>24</sub> CoCl <sub>2</sub> N <sub>8</sub> O <sub>2</sub>				(51.6)	(3.6)	(18.5)
5	[Ni(L <sup>2</sup> ) <sub>2</sub> ]Cl <sub>2</sub> [609.6]	57	211-212	3.3	51.2	3.9	18.4
	C <sub>26</sub> H <sub>24</sub> NiCl <sub>2</sub> N <sub>8</sub> O <sub>2</sub>				(51.0)	(3.8)	(18.2)
6	[Zn(L <sup>2</sup> ) <sub>2</sub> ]Cl <sub>2</sub> [616.3]	55	218-220	Dia	50.6	3.9	18.2
	C <sub>26</sub> H <sub>24</sub> ZnCl <sub>2</sub> N <sub>8</sub> O <sub>2</sub>				(50.8)	(3.5)	(18.6)

### NMR Spectra

The H NMR spectra of the free ligands taken in DMSO-d<sub>6</sub> are listed in Table 1. The free ligands exhibited signals due to all the expected protons in their expected region and have been identified from the integration curve found to be equivalent to the total number of protons deduced from their proposed structures. These were compared (Williams and Fleming 1989) with the reported signals of the known identical compounds and give further support for the compositions of the new ligands suggested by their IR and elemental analyses data.

### Magnetic Moments and UV-Visible Spectra

#### Magnetic Moments and UV-Visible Spectra

The room temperature magnetic moment of the solid cobalt(II) complexes was found at 4.2 and 4.4 B.M, indicative of three unpaired electrons per Co(II) ion in an octahedral environment (Eamshaw, 1968). The Ni(II) complexes showed  $\mu_{\text{eff}}$  values 3.1 and 3.3 B.M, corresponding to two unpaired electrons per Ni(II) ion for their ideal six-coordinated configuration (Meek et al, 1962). The Zn(II) complexes were all found diamagnetic. The electronic spectra of the Co(II) chelates showed three bands observed at 8775-8815, 17460-17825 and 30125-30110 cm<sup>-1</sup> which may be assigned to <sup>4</sup>T<sub>g</sub> → <sup>4</sup>T<sub>2g</sub>(F), <sup>4</sup>T<sub>1g</sub> → <sup>3</sup>A<sub>2g</sub>(F) and <sup>4</sup>T<sub>1g</sub> → T<sub>1g</sub> → <sup>4</sup>T<sub>1g</sub>(P) transitions respectively and are suggestive (Liehr, 1967; Carlin, 1965) of the octahedral geometry around the cobalt ions. The Ni(II) complexes exhibited three spin-allowed bands at 9875-10145, 15955-16250 and 29840-29915 cm<sup>-1</sup> assignable (Balhausen, 1962; Lever, 1986) respectively, to the transitions <sup>3</sup>A<sub>2g</sub>(F) → <sup>3</sup>T<sub>2g</sub>(F)(V<sub>1</sub>), <sup>3</sup>A<sub>2g</sub>(F)(v<sub>2</sub>) and <sup>3</sup>A<sub>2g</sub>(F) → <sup>3</sup>T<sub>2g</sub>(P)(v<sub>3</sub>) which were characteristic of their octahedral geometry. The diamagnetic zinc(II) complexes did not show any d-d bands and their spectra are dominated *only* by the charge transfer bands. The charge transfer band at 28355-28520 cm<sup>-1</sup> was assigned due to transition <sup>2</sup>E<sub>g</sub> → <sup>2</sup>T<sub>2g</sub> possibly in an octahedral environment (Figgis, 1974).

On the basis of the above observations, it is tentatively suggested that Co(II), Ni(II) and Zn(II) complexes show an octahedral geometry (Fig. 2) in which the two ligands act as tridentate and

possibly accommodate themselves around the metal atom in such the way that a stable geometry of molecule is formed.

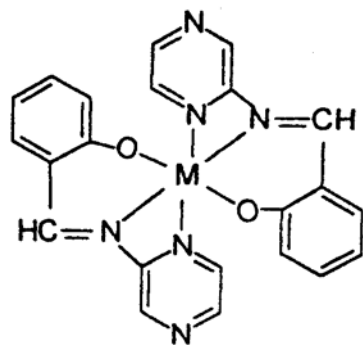
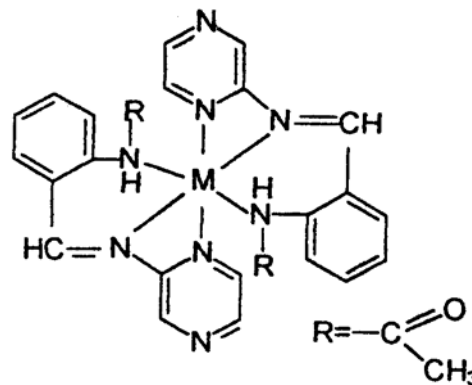
(Fig. 2A) Metal Complex of HL<sup>1</sup>(Fig. 2B) Metal Complex of L<sup>2</sup>

Table 3  
IR and UV-Visible Spectral Data of the Metal(II) Complexes

S. No.	IR (Cm <sup>-1</sup> )	λMax (cm <sup>-1</sup> )
1	1620 (HC=N), 1380 (M-O), 1315 (Py), 525 (M-N), 455 (M-O).	30125, 17825, 8775
2	1625 (HC=N), 1382 (M-O), 1320 (Py), 525 (M-N), 455 (M-O).	29915, 16250, 10145
3	1620 (HC=N), 1380 (M-O), 1320 (Py), 530 (M-N), 460 (M-O).	28520
4	3270 (NH), 1620 (HC=N), 1315 (Py), 530 (M-N).	30110, 17460, 8815
5	3265 (NH), 1625 (HC=N), 1325 (Py), 525 (M-N).	29840, 15955, 9873.
6	3262 (NH), 1620 (HC=N), 1320 (Py), 530 (M-N).	28355

#### Antibacterial Properties

The title Schiff-base ligands and their metal dictates were evaluated for their antibacterial activity against bacterial species *Escherichia coli* (a), *Staphylococcus aureus* (b) and *Pseudomonas aeruginosa* (c) The compounds were tested at a concentration of 30 µg/0.01 mL in DMF solution using the paper disc diffusion method. The susceptibility zones were measured in diameter (mm) and the results are reproduced in Table 4. The susceptibility zones measured were the clear zones around the discs killing the bacteria.

All the Schiff bases and their complexes individually exhibited varying degrees of inhibitory effects on the growth of the tested bacterial species. The antibacterial results evidently show that the activity of the Schiff-base compounds became more pronounced when coordinated to the metal ions. It is suggested that in the chelated complex, the positive charge of the metal is partially shared with the donor atoms present in the ligands and there is  $\pi$ -electron delocalization over the whole chelate ring. This in turn increases the lipophilic character of the metal chelate and favors its permeation through the lipid layers of the bacterial membranes. Apart from this, other factors such as solubility, conductivity and dipole moment (influenced by the presence of metal ions) may also be the possible reasons for increasing this activity.

**Table 4**  
Antibacterial Activity Data of the Schiff-bases and Metal(II) complexes

Schiff- base/Complex	Microbial Species		
	a	B	c
HL <sup>1</sup>	++	+	++
L <sup>2</sup>	++	++	+
1	+++	+++	++
2	+++	++	+++
3	+++	+++	+++
4	++++	+++	+++
5	+++	+++	+++
6	++++	++	+++

A = *Escherichia coli*, B = *Staphylococcus aureus*, C = *Pseudomonas aeruginosa*

Inhibition zone diameter mm (% inhibition): +, 6-10 (27-45%); ++, 10-14 (45-64%); +++, 14-18 (64-82%); +++++, 18-22 (82-100%). Percent inhibition values are relative to inhibition zone (22 mm) of the most active compound with 100% inhibition.

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