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Antifungal and Antimicrobial Activities of New Schiff Base and Its Metal Complexes

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The *in vitro* antibacterial and antifungal activities of Schiff base derived from the reaction of phthaldehyde with 4-amino-2,3-dimethyl-1-phenyl-3-pyrozolin-5-one and its Co(II), Cu(II) and Ni(II) metal complexes were investigated. The structure of the Schiff base was confirmed by ^{1}H NMR. Schiff base and its metal complexes were evaluated for both their *in vitro* antibacterial activity against *Klebsiella pneumoniae* FMC 5, *Enterococcus cloacae* ATCC 13047, *Escherichia coli* ATCC 8739, *Bacillus megaterium* DSM 32, *Staphylococcus aureus* Cowan 1, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatis* CCM 2067, *Pseudomonas aeruginosa* ATCC 27853 and their *in vitro* antifungal activity against *Candida albicans* ATCC 1023, *Kluyveromyces fragilis* A 230, *Rhodotorula rubra* by the disc diffusion method. The antimicrobial activities tended to decrease with the increasing size of the amino acid residues.

Key Words: Schiff base complex, Antibacterial activity, Antifungal activity.

INTRODUCTION

Transition metal complexes are important in many areas of science, including catalysis, medicine (diagnosis and therapy), design of high value materials, analytical chemistry and as model compounds which the structure and function of metalloproteins[1-4]. The metal oxidation state, the type and number of donor atoms, as well as their relative disposition within the ligand, are major factors determining structure-activity relationship of the metal complexes[5,6].

The preparation of new ligands is perhaps the most important step in the development of metal complexes with unique properties and novel reactivity. In recent years, the chemistry of coordination compounds display rapid development in diverse disciplines due to the possible biological applications of these new compounds. Metal chelates play an essential role in the chemistry of living organisms and a large number of metal proteins and other metal complexes of biological importance have been studied[7,8].

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Schiff bases derived from an amine and aldehydes are an important class of ligands that coordinate to metal ions *via* azomethine nitrogen and have been studied extensively[9-12]. Many papers indicate that the environment around the metal ion and the conformational flexibility of the ligands are the most important facts because they allow metalloproteinase to carry out a specific biological function. The flexibility of Schiff base ligands can be improved by hydrogenation of their C=N bonds, they should thus coordinate metal ions more easily. For these reasons, reduced Schiff base have recently gained considerable attention[13]. Schiff bases of 4-aminoantipyrine and its complexes are known for their variety of applications in the area of catalysis, clinical and pharmacology[14]. Antipyrine and its derivatives posses antibacterial and antitumor activities[15]. New kinds of chemo-therapeutic agents containing among biochemists and of those amino pyridines are commonly administered intravenously to detect liver disease in clinical treatment[16].

In this studies, the syntheses, characterization and biological activities Schiff base ligand and its transition metal complexes are reported. The ligand in this study was synthesized by the reaction of phthaldehyde and glyoxal with 4-amino-2,3-dimethyl-1-phenyl-3-pyrazolin-5-one. The ligand may be represented as shown in Fig. 1. Its complexation ability with Co(II), Ni(II) and Cu(II) salts is also examined. The ligand and its complexes were examined antibacterial and antifungal activities against different bacteria and fungi.

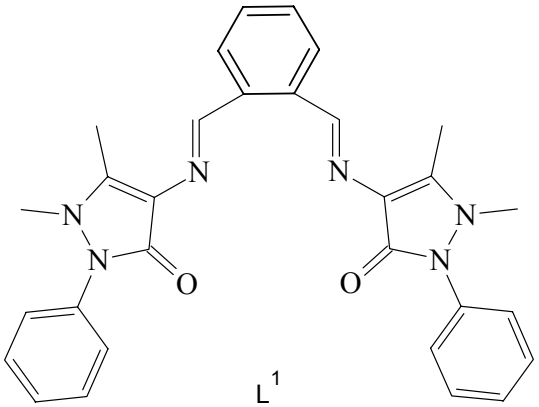


Fig. 1. Proposed structure of L^1 Schiff base ligand

EXPERIMENTAL

The metal salts ($CoCl_2 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$) and phthaldehyde were obtained from Fluka. 4-Amino-2,3-dimethyl-1-phenyl-3-pyrozolin-5-one was obtain from Merck. Solvents were analytical grade and were purified by standard procedures.

Melting points were determined with a digital melting point apparatus using capillary technique. The elemental analyses for ligand and its metal complexes were carried out at the Leuco 632 analyzer. ^{1}H NMR spectra were recorded on a Varian XL-200 NMR instrument. TMS was used as internal standard and deuteriated DMSO as solvent. IR spectra were obtained using KBr discs (4000-400 cm^{-1}) on a Shimadzu 8300 FTIR spectrophotometer. The electronic spectra in the 200-900 nm range were obtained using DMSO on a Shimadzu UV-160 A spectrophotometer. Molar conductances of the Schiff base ligand and its transition metal complexes were determined in MeOH (*ca.*10^{-3} M) at room temperature using a Jenway Model 4070 conductivity meter.

Cultures: *Klebsiella pneumoniae* FMC 5, *Enterococcus cloacae* ATCC 13047, *Escherichia coli* ATCC 8739, *Bacillus megaterium* DSM 32, *Staphylococcus aureus* Cowan 1, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatis* CCM 2067 and *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 1023, *Kluyveromyces fragilis* A 230 and *Rhodotorula rubra* microorganisms were used.

Synthesis of 4,4'-(1E,1'E)-(1,2-phenylene*bis*(methan-1-yl-1-ylidene))*bis*-(azan-1-yl-1-ylidene)*bis*(1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one) (L^1): Phthaldehyde (1.34 g, 0.01 mol) and the 100 mL suspension of amine compound (4.06 g, 0.02 mol) were stirred until a thick precipitate appeared. Stirring was continued for 24 h and then the brownish product was collected by vacuum filtration and dried overnight in air. ^{1}H NMR (DMSO-d_6, δ ppm): 9.73 (s, 2 H, CH=N), 8.03-7.12 (m, 14 H, Ar-H), 3.23 (s, 6 H, C-CH_3), 2.13 (s, 6 H, N-CH_3).

Synthesis of the metal complexes: The Co(II), Cu(II) and Ni(II) complexes of Schiff base ligand were prepared according to the reported method[17].

The complexes were synthesized by heating equimolar amounts of the ligand and the metal salts in ethanol for 24 h. The solid complexes were separated by filtration and washed with petroleum ether. Elemental analysis data, colour and yield for the complexes are given in Table-1.

TABLE-1
ANALYTICAL DATA AND PHYSICAL PROPERTIES OF SCHIFF BASE LIGAND AND ITS METAL COMPLEXES

Compound (m.f.)	m.w. (m.p., °C)	Colour	Λ / (Yield, %)	Elemental analysis (%): Found (Calcd.)		
				C	H	N
L^1 (I)	533.60	Orange	1.10	69.70	5.86	18.34
($C_{31}H_{31}N_7O_2$)	(215)		(81)	(69.77)	(5.86)	(18.37)
[Co(L^1)(Cl)].Cl.3H_2O (III)	768.55	Brown	10.25	51.19	4.96	13.34
($C_{34}H_{38}N_8O_5Cl_2Co$)	(> 250)		(67)	(51.22)	(5.01)	(13.94)
[Cu(L^1)(Cl)].Cl.3H_2O (V)	773.17	Dark brown	11.20	50.28	4.93	13.71
($C_{34}H_{38}N_8O_5Cl_2Cu$)	(>250)		(72)	(50.89)	(4.98)	(13.85
[Ni(L^1)(Cl)].Cl.3H_2O (VII)	768.31	Dark brown	13.21	51.17	5.03	13.66
($C_{34}H_{38}N_8O_5Cl_2Ni$)	(>250)		(75)	(51.24)	(5.02)	(13.94)

Biological studies: New Schiff base and its complexes L^1, [Co(L^1)], [Cu(L^1)] and [Ni(L^1)] were evaluated for both their *in vitro* antibacterial activity against *Klebsiella pneumoniae* FMC 5, *Enterococcus cloacae* ATCC 13047, *Escherichia coli* ATCC 8739, *Bacillus megaterium* DSM 32, *Staphylococcus aureus* Cowan 1, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatis* CCM 2067, *Pseudomonas aeruginosa* ATCC 27853 and their *in vitro* antifungal activity against *Candida albicans* ATCC 1023, *Kluyveromyces fragilis* A 230, *Rhodotorula rubra* by the disc diffusion method. All the bacteria mentioned above were incubated in Nutrient Broth (NB) (Difco) at 37 ± 0.1 °C for 24 h and the yeasts were incubated in Sabouraud Dextrose Broth (SDB) (Difco) at 25 ± 0.1 °C for 48 h. The bacteria and yeasts (prepared as above) were injected into petri dishes (9 cm) in the amount of 0.01 cm^3 ($10^5/cm^3$ for the bacteria and $10^3/cm^3$ for the fungi), 15 mL of Mueller Hinton Agar (MHA, Oxoid) and Sabouraud Dextrose Agar (SDA) (sterilized in a flask and cooled to 45-50 °C) were homogenously distributed onto the sterilized petri dishes[18].

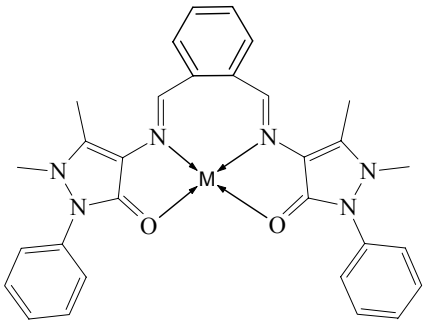
Schiff base and its metal complexes were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Shüll No: 2668, Germany) in the amount of 30 µL. The compounds to be tested were dissolved in acetone to a final concentration of 2000 ppm and soaked in filter paper. Discs injected with complexes were located on the solid agar medium by pressing slightly. After petri dishes so obtained were placed at 4 ºC for 2 h, plates inoculated with fungi were incubated at 25 ± 0.1 ºC for 24 h. At the end of the period, inhibition zones formed on the food medium were evaluated is millimeters[18]. These studies works were performed in duplicate. Ampicillin, cefodizime, cefuroxime, cephalothin, oxacillin (bioanalyse) and nystatin (oxoid) were used as standards.

RESULTS AND DISCUSSION

In this present work, 4,4'-(1E,1'E)-(1,2-phenylene*bis*(methan-1-yl-1-ylidene))-*bis*(azan-1-yl-1-ylidene)*bis*(1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one) (L^1) Schiff base ligand was prepared by the reaction of phthaldehyde with 4-amino-2,3-dimethyl-1-phenyl-3-pyrozolin-5-one and its Co(II), Cu(II) and Ni(II) metal complexes have also been prepared. The impurities were checked by TLC.

The results of the elemental analyses of the ligand and complexes are in agreement with the chemical formulae. The ligand is stable at room temperature and soluble in common organic solvents such as DMSO, DMF, EtOH and MeOH. The complexes are also stable at room temperature. Based on the elemental analyses, spectroscopic characterization, these mononuclear complexes are presumed to have the coordination environment shown in Fig. 2. Single crystals of the compounds could not be isolated from any organic solution, thus no definite structures can be described.

New Schiff base and its complexes, L^1, [Co(L^1)], [Cu(L^1)] and [Ni(L^1)] were evaluated for both their *in vitro* antibacterial activity against *Klebsiella pneumoniae* FMC 5, *Enterococcus cloacae* ATCC 13047, *Escherichia coli* ATCC 8739, *Bacillus megaterium* DSM 32, *Staphylococcus aureus* Cowan 1, *Micrococcus luteus* LA



M = Co(II), Cu(II) and Ni(II)

Fig. 2. Proposed structures of the L^1 complexes

2971, *Mycobacterium smegmatis* CCM 2067, *Pseudomonas aeruginosa* ATCC 27853 and their *in vitro* antifungal activity against *Candida albicans* ATCC 1023, *Kluyveromyces fragilis* A 230, *Rhodotorula rubra* by the disc diffusion method. Analytical data of ligand and their complexes are presented in Table-1.

All the complexes are insoluble in EtOH and MeOH, but are soluble in DMSO and DMF to give stable solutions at room temperature. The molar conductances of the complexes are given in Table-1. Equivalent conductance of complexes carried out in 1×10^{-3} M methanol solutions are in the range 10.25-13.21 (ohm^{-1} cm^2 mol^{-1}) suggesting that non-electrolyte nature of the complexes.

^{1}H NMR spectra: The ^{1}H NMR spectra of the ligand, L^1 exhibit singlet signals signals in the 8.10-7.12 ppm range can be attributed to the aromatic ring protons[19]. The ligand exhibit singlet signal in the 9.73 ppm which is attributed to the azomethine group proton[20]. The singlets in the 3.23-3.22 and 2.15-2.13 ppm ranges may also be attributed to the C-CH_3 and N-CH_3 group protons, respectively[21,22].

Infrared spectra: The characteristic infrared data of the Schiff base ligand and its metal complexes listed in Table-2. In the ligands and complexes spectra, both ligands and complexes exhibit bands at 3062-3051 cm^{-1} that are assignable to ν(Ar-CH)[23]. The infrared spectral data of the Schiff base ligands L^1 and L^2 show a very strong band at 1654 and 1652 cm^{-1} which may be attributed to the ν(C=O) stretching vibration of the antipyrine ring. On complexation these bands have shifted to the lower region 1631-1610 cm^{-1} showing that carbonyl oxygens are involved in coordination[24].

TABLE-2
CHARACTERISTIC INFRARED BANDS (cm^{-1}) OF THE SCHIFF BASE LIGAND AND ITS METAL COMPLEXES, L^1, [Co(L^1)], [Cu(L^1)], [Ni(L^1)]

Compound	ν(ArC-H)	ν(C=O)	ν(CH=N)	ν(M–O)	ν(M–N)
L^1	3062	1652	1589	-	-
[Co(L^1)]	3057	1610	1565	597	421
[Cu(L^1)]	3054	1625	1578	572	435
[Ni(L^1)]	3056	1623	1607	583	442

Also the medium intensity bands at 1625 and 1618 cm^{-1} assignable to the azomethine ν(C=N) stretching of L^1 and L^2 are shifted to the region 1610-1593 cm^{-1}, respectively, thereby indicating coordination of azomethine nitrogen atoms[25]. The infrared spectra of Schiff base complexes are similar. For the metal complexes, new weak bands in the 607-572 cm^{-1} and 442-420 cm^{-1} range may be attributed to ν(M-O) and ν(M-N), respectively[26].

UV-Visible spectra: The electronic absorption spectral data for ligands and complexes are given in Table-3. Electronic spectrum of ligands showed a band at *ca*. 390 nm due to the n-π* transition of azomethine group. On complexation this band has shifted to lower wavelength, suggesting the coordination of azomethine nitrogen[27]. The UV spectra of the metal complexes of the ligands in EtOH solution showed a high intensity broad band in the 450-401 nm range which is tentatively assigned to ligand (π)- metal (d) charge-transfer, while the weaker ligand field bands may be obscured by such transitions[28]. The spectra of all the complexes contain an absorption band in the 741-615 nm range may be assigned to the *d-d* transition of metal ions. The Co(II) complexes show bands at 641 and 659 nm.

TABLE-3
ELECTRONIC SPECTRAL DATA OF THE SCHIFF BASE LIGAND AND ITS METAL COMPLEXES

Compound	λ_{max} (nm)
L^1	223, 272, 334, 391
$[Co(L^1)]$	211, 281, 333, 399, 455, 641
$[Cu(L^1)]$	236, 278, 335, 363, 425, 721
$[Ni(L^1)]$	271, 330, 357, 375, 615

Biological activity: The antibacterial and antifungal activity of the four new compounds was tested by using disc diffusion method. The antibacterial and antifungal activities of the new compounds against eight bacteria, namely *Klebsiella pneumoniae, Enterococcus cloacae, Escherichia coli, Bacillus megaterium, Staphylococcus aureus, Micrococcus luteus, Mycobacterium smegmatis, Pseudomonas aeruginosa* and three fungi, namely *Candida albicans, Kluyveromyces fragilis, Rhodotorula rubra*, are presented in Table-4. Ampicillin, cefodizime, cefuroxime, cephalothin, oxacillin (bioanalyse) and nystatin (oxoid) were taken as the standards reference antibiotics for antibacterial and antifungal activities (Table-5). The antibacterial and antifungal activities of standard antibiotics gave 45 and 8 mm inhibition zones for fungi and bacteria.

The results showed that the Schiff base ligands L^1 exhibits slight activity against all species of fungi, *Candida albicans, Kluyveromyces fragilis, Rhodotorula rubra* and all species of bacteria, *Klebsiella pneumoniae, Enterococcus cloacae, Escherichia coli, Bacillus megaterium, Staphylococcus aureus, Micrococcus luteus, Mycobacterium smegmatis* and *Pseudomonas aeruginosa*, on the biological activities of the standard antibiotics. The Schiff base ligand L^1 has the highest effect against *Escherichia coli*

TABLE-4
ANTIMICROBIAL STUDIES OF SCHIFF BASE LIGAND AND ITS METAL COMPLEXES (DIAMETER OF INHIBITION ZONE (mm); CONCENTRATION: 2000 ppm, 30 µL/ disc)

Compd.	Bacteria, inhibition zone (mm)								Fungi, inhibition zone (mm)		
	A	B	C	D	E	F	G	H	I	J	K
L^1	8	0	10	7	7	8	8	7	8	8	7
$[Co(L^1)]$	0	0	0	0	0	0	0	0	7	10	7
$[Cu(L^1)]$	0	0	0	0	0	0	0	0	10	14	15
$[Ni(L^1)]$	0	0	0	0	0	0	0	0	9	12	7

A= *Klebsiella pneumoniae*, B = *Enterococcus cloacae*, C = *Escherichia coli*, D = *Bacillus megaterium*, E = *Staphylococcus aureus*, F = *Micrococcus luteus*, G = *Mycobacterium smegmatis*, H = *Pseudomonas aeruginosa*, I = *Candida albicans*, J = *Kluyveromyces fragilis*, K = *Rhodotorula rubra*,

TABLE-5
ANTIMICROBIAL ACTIVITIES OF SOME STANDARD ANTIBIOTICS [DIAMETER OF INHIBITION ZONE (mm)]

Compd.	Bacteria, inhibition zone (mm)								Fungi, inhibition zone (mm)		
	A	B	C	D	E	F	G	H	I	J	K
Amp 10 µg	14	15	15	20	14	15	15	0	0	0	0
Cef 30 µg	12	13	32	0	12	0	0	0	0	0	0
Cefu 30 µg	0	0	20	0	0	0	0	0	0	0	0
Cep 30 µg	0	0	45	0	45	0	13	10	0	0	0
Oxa 1 µg	0	8	29	0	0	0	0	0	0	0	0
Nys 30 µg	0	0	0	0	0	0	0	0	18	16	18

A= *Klebsiella pneumoniae*, B = *Enterococcus cloacae*, C = *Escherichia coli*, D = *Bacillus megaterium*, E = *Staphylococcus aureus*, F = *Micrococcus luteus*, G = *Mycobacterium smegmatis*, H = *Pseudomonas aeruginosa*, I = *Candida albicans*, J = *Kluyveromyces fragilis*, K = *Rhodotorula rubra*,
Amp = Ampicillin, Cef = Cefodizime, Cefu = Cefuroxime, Cep = Cephalothin,
Oxa = Oxacillin, Nys = Nystatin (Oxoid)

in comparison to other bacteria. All the complexes showed moderate activity against all tested fungi, but no activity against all tested bacteria. Among the complexes tested, the $[Cu(L^1)]$ showed higher antifungal activity than the other complexes. The $[Ni(L^1)]$ complex showed higher antifungal activity than $[Co(L^1)]$ complex.

The variation in the activity of different metal complexes against different microorganisms depends on either the impermeability of the cells of the microbes or the differences in ribosomes in microbial cells[29].

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