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Synthesis, Structural elucidation, Pharmacological evaluation of Metal complexes with 2-Pyrazoline derivatives and Metalation effect on the Antimicrobial activity

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Abstract

1-Acetyl-5-(4-chlorophenyl/4-nitrophenyl)-3-(4-aminophenyl)-2-pyrazolines and their metal complexes have been synthesized and are being characterized through molecular weight determination, molar conductance, magnetic susceptibility, and spectroscopic techniques. The IR data have indicated that the coordination of ligands with the metal ions (Fe^{2+}, Co^{2+}, and Ni^{2+}) occurs via azomethine nitrogen and carbonyl oxygen atoms. Microanalytical data, along with electronic spectral analysis and magnetic moment studies, suggested an octahedral geometry around the metal ions with a 1:2 metal-ligand ratio. Additionally, the complexes demonstrated significantly enhanced in vitro antimicrobial activity compared to the free ligand. The anti-microbial activities of these derivatives have been studied by screening them against bacteria such as Bacillus subtilis, Staphylococcus aureus, and Escherichia coli and fungi such as Penicillium chrysogenum and Aspergillus niger by the Agar Well Diffusion method.

Keywords: Antimicrobial activity, Metal complexes, Pyrazolines.

Introduction

Pyrazolines are weak bases and good chelating agents due to the presence of donor nitrogen atoms in the nucleus. This property has been utilized in the preparation of many of their coordination compounds with different metal ions. Metal complexes derived from pyrazolines have attracted considerable interest not only due to their extensive coordination chemistry but also to their catalytic and biological properties. In order to achieve the above impending challenges, we are trying to design new metal-based drugs having heterocyclic ligand systems. Among the general heterocyclic systems, the azo-linked molecules are mostly used in preparing transition metal chelates due to their versatile properties. Metal complexes of pyrazoline exhibit biological activities such as anti-inflammatory[1], antiviral[2], antibacterial[3], antifungal[4], antitubercular[5], antioxidant[6] activities, etc.

Methodology

Physico-chemical measurements: The ^{1}H NMR spectra in $CDCl_3$ were recorded on the DELTA2-NMR (400 MHz) spectrometer in DMSO-D6 using TMS as the internal reference. IR spectra in universal mode on KBr plates (λmax in cm^{-1}) were obtained using a Bruker ALPHA FTIR infrared spectrophotometer within the range of 4000–500cm^{-1}. Conductance values are being measured in dry DMSO at a concentration of 10^{-3}M using a digital conductivity meter, model DELUXE 60. Molecular weight was determined by the Cryoscopic method in dry DMSO. Magnetic susceptibilities were measured on the Lakeshore 7410S VSM model, (at 25+-1^0C) and C, H, and N analyses were performed on an UNICUBE model. Metals were estimated by the atomic absorption spectrophotometer Element AS, model iCAP-RQ.

Synthesis of pyrazoline ligands: The pyrazoline ligands were synthesized by two methods: Synthesis of 3-(4-chlorophenyl/4-nitrophenyl)-1-(4-aminophenyl)-2-propene-1-one (1): Conventional method: 4-Aminoacetophenone (0.01 mol) was dissolved in 5% methanolic sodium hydroxide (30 mL) with constant stirring, and 4-chlorobenzaldehyde or 4-nitrobenzaldehyde (0.01 mol) was added dropwise at 50°C. The mixture was stirred for 10 minutes or 2 hours, respectively, depending on the aldehyde used. After removing the stirrer, the reaction mixture was cooled, poured onto ice-cold distilled water, neutralized with dilute sulfuric acid, and filtered. The solid was washed with cold distilled water, dried in a vacuum oven, and purified by recrystallization from methanol to obtain the chalcones.

Microwave method: Equimolar amounts of both reactants were taken in a conical flask with 5mL of alcohol and heated in a microwave for 30 seconds at a low-medium temperature. Subsequently, 5mg of NaOH was added, and the mixture was microwaved for an additional 2 minutes. The reaction mixture was then diluted with water, acidified with concentrated sulfuric acid, and the resulting solid was washed with water, recrystallized from methanol, and dried under reduced pressure.

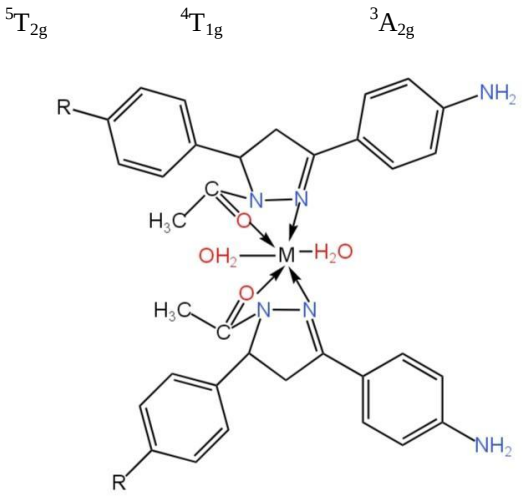
Synthesis of 1-Acetyl-5-(4-chlorophenyl/4-nitrophenyl)-3-(4-aminophenyl)-2-pyrazolines: The reaction was conducted in a 250mL double-necked round-bottom flask equipped with a condenser. A solution of 3-(4-chlorophenyl/4-nitrophenyl)-1-(4-aminophenyl)-2-propene-1-one (0.01 mol) in 30mL of glacial acetic acid was refluxed with an excess of hydrazine hydrate (2.5mL) for 2 hours. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was cooled and poured onto ice water. The resulting solid was filtered, washed with distilled water, dried under vacuum, and recrystallized from methanol.

Synthesis of Metal complexes: A mixture of metal salt—ferrous sulfate, cobalt chloride, or nickel chloride—and the ligand (in a 1:2 ratio) in an ethanolic medium was refluxed for 30 minutes, 50 minutes, or 1 hour, respectively. Metal salts (0.002mol) were dissolved in a minimal amount of water and slowly added to the ethanolic ligand solution (0.004 mol) with continuous stirring. Colored products (yellow, light brown, or brown) formed upon standing and cooling. The precipitates were filtered, washed multiple times with aqueous ethanol, and dried in a vacuum oven at 60–70°C.

Results and Discussion

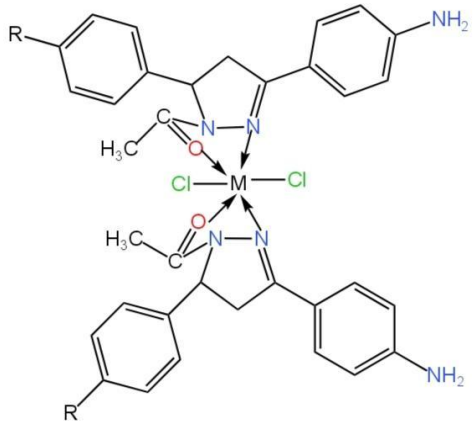
Substituted 3-(4-chlorophenyl/4-nitrophenyl)-1-(4-aminophenyl)-2-propene-1-one was synthesized via the condensation of 4-aminoacetophenone and substituted (4-chloro/4-nitro) aldehydes in a methanolic sodium hydroxide solution. These compounds were assigned a trans configuration based on the coupling constant (16 Hz) observed between two olefinic protons, appearing at 7.40 and 8.10 ppm in the ^{1}H NMR spectra. The reaction of (1) with hydrazine hydrate in glacial acetic acid resulted in the formation of ligands L2 and L5. The synthesis of ligands involved an initial nucleophilic attack at the carbonyl group, producing unstable α, β-unsaturated hydrazones. These intermediates underwent intramolecular rearrangement at the double bond, followed by *in situ* acylation due to the presence of glacial acetic acid as the solvent. In these ligands, the trans C_4-H proton was observed up field (3.08–2.99ppm) compared to its cis analogue (3.04–3.13ppm).

The metal complexes obtained were colored and stable under atmospheric conditions. These complexes were insoluble in common organic solvents such as ethanol, acetone, ether, and benzene but dissolved in polar solvents like DMSO. The low molar conductance values in dry DMSO indicated their non-electrolytic nature. Elemental analyses, magnetic moment studies, and FTIR spectra confirmed the octahedral geometry around the Fe(II) (Figure-1), Co(II), and Ni(II) (Figure-2) complexes, consistent with previously reported findings[7]. These metal complexes generally exhibited high spin states, attributed to the additional stability of half-filled and fully filled 3d sub shells. The observed effective magnetic moments further supported the high spin configuration of the metal complexes.
Fe^{+2}=4.90B.M., Co^{+2}=3.87B.M., Ni^{+2}=2.83B.M.



M=Fe^{+2}

Figure-1: Octahedral structure of iron complex.



M=Co^{+2} & Ni^{+2}

Figure-2: Octahedral structures of cobalt & nickle complex.

Infrared spectra: The infrared spectra of the complexes exhibited a shift in the ν(C=N) mode from 1521–1565 cm^{-1} in the ligands to 1500–1540 cm^{-1} in the complexes (Table-1). Additionally, the ν(N-N) mode displayed a positive shift of 10–20 cm^{-1}. The negative shift in ν(C=N) and the positive shift in ν(N-N) confirmed the coordination of the azomethine nitrogen of the pyrazoline group to the metal ion. The involvement of the carbonyl oxygen in bonding was evidenced by a downward shift in the ν(C=O) mode from 1636–1688 cm^{-1} in the ligands to 1600–1660 cm^{-1} in the complexes. Meanwhile, the thiophene ring stretching vibrations remained unchanged in the spectra of the metal complexes.

New absorption bands in the far-infrared region, observed at 618–721 cm^{-1} and 533–539 cm^{-1}, were assigned to M-N and M-Cl stretching vibrations, respectively. The iron complexes also showed a broad band near 3000–3500cm^{-1}, indicative of coordinated water molecules. The splitting of the band in the 1030–1255cm^{-1} range suggested unidentate coordination of

SO_4^{2-} ions. These IR findings confirmed that the ligands coordinated to the metal ions through azomethine nitrogen and carbonyl oxygen. Elemental analyses of the complexes were consistent with the proposed formulae and molecular weights (Table-2).

Antimicrobial Activity: The Agar Well Diffusion method, as described by Perez and Anesini[8], was used for the in-vitro antimicrobial assay. The synthesized ligands and their metal complexes were tested for antimicrobial activity against two economically significant phytopathogenic fungi, *Penicillium chrysogenum* and *Aspergillus niger*, as well as three bacterial strains: the gram-positive *Bacillus subtilis*, *Staphylococcus aureus*, and the gram-negative *Escherichia coli* (Table-3).

The antimicrobial activity data indicated that all the metal complexes exhibited greater biological activity compared to the free ligands. The enhanced activity of the metal complexes could be attributed to the influence of metal ions on normal cellular processes. Chelation is known to increase the lipophilic nature of the metal complexes, which likely disrupts the cell membrane's permeability barrier, leading to interference with cellular functions. Among the metal complexes, the cobalt (II) complex (Figure-3, 4) demonstrated the highest activity, surpassing the activities of the Ni(II) and Fe(II) complexes.

Table-1: Infrared spectral characteristics of metal complexes.

Compounds	(C=O)	(C=N)	(C=C)	(C-N)	(N-N)	(M-O)	(M-N)	(M-Cl)
$[Fe(HL_5)_2(H_2O)SO_4]$	1636	1521	1445	1342	843	618	539	-
$[Co(HL_2)_2Cl_2]$	1621	1534	1471	1316	831	721	533	295
$[Ni(HL_2)_2Cl_2]$	1688	1544	1452	1361	851	707	524	317

Table-2: Magnetic, physical, and analytical data of metal complexes

Compounds	M.F.	M.W.	Yield %	C	H	N	Metal	$\mu_{eff.}$ (B.M.)
$[Fe(HL_5)_2(H_2O)SO_4]$	$C_{34}H_{34}FeN_8O_{11}S$	816 (818)	84	49.4 (49.8)	3.8 (4.1)	13.3 (13.6)	6.4 (6.8)	4.3 (4.1)
$[Co(HL_2)_2Cl_2]$	$C_{34}H_{32}CoCl_4N_6O_2$	761 (757)	88	53.2 (53.8)	4.1 (4.2)	10.8 (11.0)	7.1 (7.7)	4.0 (4.1)
$[Ni(HL_2)_2Cl_2]$	$C_{34}H_{32}NiCl_4N_6O_2$	755 (756)	80	53.7 (53.9)	4.0 (4.2)	10.3 (11.1)	7.4 (7.7)	2.5 (2.1)

Table-3: Inhibition zone (mm) of metal complexes via diffusion method.

Compounds	Bacteria									Fungi							
	Bacillus subtilis			S. aureus			E. coli			P. chrysogenum				Aspergillus niger			
	50 mg/ L	100 mg/ L	150 mg/ L	50 mg/ L	100 mg/ L	150 mg/ L	50 mg/ L	100 mg/ L	150 mg/ L	25 mg/ L	50 mg/ L	75 mg/ L	100 mg/ L	25 mg/ L	50 mg/ L	75 mg/ L	100 mg/ L
$[Fe(HL_5)_2(H_2O)SO_4]$	0	0	0	11	13	16	8	11	14	7	8	10	11	7	8	9	11
$[Co(HL_2)_2Cl_2]$	0	0	0	8	11	15	13	17	21	13	15	16	21	9	10	11	12
$[Ni(HL_2)_2Cl_2]$	0	0	0	8	11	14	10	13	17	8	9	10	12	8	9	10	11
Standard drug	36	28	30	31	27	24	32	33	35	20	21	23	24	8	18	21	22

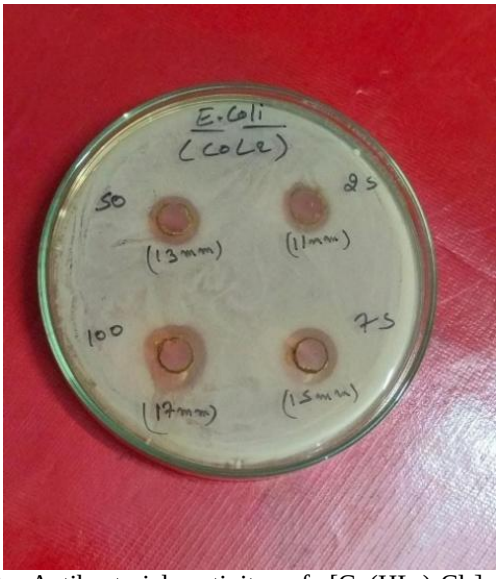


Figure-3: Antibacterial activity of $[Co(HL_2)_2Cl_2]$ complex against E. coli.

Figure-4: Antifungal activity of $[Co(HL_2)_2Cl_2]$ complex against Penicillium.

Conclusion

The observed activities of metal complexes showed that the cobalt complexes had the greatest activity as compared to Ni(II) and Fe(II) complexes and in comparison of Ni(II) and Fe(II) complexes, Ni(II) complexes more active than Fe(II) complexes.

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