Environmentally Friendly Schiff Base–Metal Complexes Promising Agents against Pathogens and Cancer Cells

Gajbhiye R. G.
Department of Chemistry, Vidya Vikas Arts, Commerce & Science College Samudrapur, Wardha, MS, India.

***Abstract*—A mixed ligand complex was synthesized using the Schiff base ligand p-Methoxy Isonitrosoacetophenone (p-MINAP), prepared by the reaction of p-Methoxyacetophenone with n-amyl nitrite, as the primary ligand, and 1,10-Phenanthroline (1,10-Phen) as the secondary ligand. The Schiff base ligand and the resulting mixed ligand complex were characterized through elemental analysis, IR, UV-Visible spectroscopy, ^{1}H NMR, molar conductance, and magnetic moment studies.**

The mixed ligand complex was found to have the general formula [M(p-MINAP)(1,10-Phen)], with an octahedral geometry around the metal center. The synthesized Schiff base (p-MINAP), the 1,10-Phenanthroline ligand, and their mixed ligand complex were evaluated for antibacterial and antifungal activities against both Gram-positive and Gram-negative bacteria, as well as fungi. The results revealed significant antimicrobial activity of the synthesized compounds.

***Index Terms*—Schiff base, p-MINAP, 1,10-Phenanthroline, Mixed ligand complex.**

I. INTRODUCTION

Mixed ligand complexes of transition metal ions play a significant role in coordination chemistry due to their diverse biological, photochemical, medical, and electromagnetic applications [1–2]. Over the past few decades, transition metal complexes have gained importance in medicinal chemistry, particularly in the treatment of various diseases, making this an active area of research in coordination chemistry [3]. The unique ability of transition metals to exist in multiple oxidation states and to coordinate with a wide range of ligands makes them crucial in the field of medicinal inorganic chemistry [4–5].

Schiff base ligands and their metal complexes have been widely studied for their versatility. These compounds serve various roles, including metal bio-site modeling, mimicking reaction centers of metalloenzymes, and functioning as nonlinear optical materials, catalysts for organic reactions, and luminescent materials [6–7]. Moreover, Schiff base complexes exhibit a broad spectrum of biological activities, such as anticancer, antifungal, antibacterial, antiviral, and antiparasitic effects, contributing significantly to advancements in medicinal and coordination chemistry [8–13].

Recent research has focused on mixed ligand complexes involving Schiff bases and nitrogen-containing heterocyclic amines. Nitrogen-donor ligands are among the most effective chelators for transition and post-transition metal ions, forming highly stable complexes. In particular, ligands such as 1,10-phenanthroline (1,10-Phen), which contain aromatic and heteroaromatic rings, have demonstrated enhanced chemical properties along with therapeutic, pharmacological, and DNA cleavage activities [14–18].

In the present study, a novel Schiff base ligand, p-Methoxy Isonitrosoacetophenone (p-MINAP), was synthesized from p-Methoxyacetophenone and n-amyl nitrite. A mixed ligand complex was then prepared using p-MINAP as the primary ligand and 1,10-phenanthroline as the secondary ligand. The synthesized compounds were characterized using elemental analysis and various spectroscopic techniques. Additionally, their antibacterial and antifungal activities were investigated [19].

II. EXPERIMENTAL

Materials and Methods

All chemicals used in this study were of analytical reagent (A.R.) grade. The starting materials included p-methoxyacetophenone, n-amyl nitrite, and 1,10-phenanthroline. The metal salt used was copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$), obtained from Sigma. Ethanol (95%) and N,N-dimethylformamide (DMF) were used as solvents.

Synthesis of Schiff Base Ligand (p-MINAP):

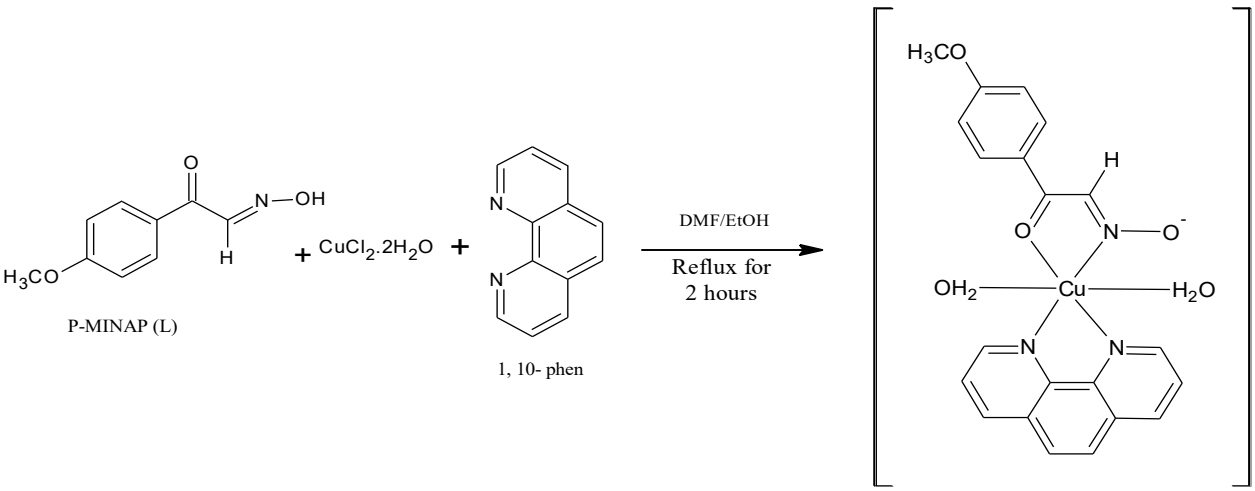
To prepare the Schiff base ligand p-Methoxy Isonitrosoacetophenone (p-MINAP), 14.5 g of sodium metal was dissolved in 280 mL of absolute ethanol. To this solution, 60 mL of n-amyl nitrite was added slowly with continuous cooling, followed by the gradual addition of 70 mL of p-methoxyacetophenone. The resulting reaction mixture was transferred to a well-stoppered bottle and kept in a refrigerator for three days.

After this period, the brown sodium salt that formed was filtered and air-dried. The dried salt was then dissolved in a minimal amount of ice-cold water and acidified with a calculated amount of glacial acetic acid. The resulting precipitate of p-methoxy isonitrosoacetophenone was collected by suction filtration and dried under vacuum. The crude product was recrystallized from benzene, yielding pure p-MINAP with a melting point of 96 °C.

Synthesis of Mixed Ligand Complex:

The mixed ligand complex was synthesized by combining copper (II) chloride dihydrate (0.3409 g, 2 mmol) with p-MINAP (0.36 g, 2 mmol) and 1,10-phenanthroline (0.36 g, 2 mmol) in a 1:1:1 molar ratio. p-MINAP was dissolved in 50 mL of DMF, while 1,10-phenanthroline was dissolved in 20 mL of ethanol. The metal salt was added to this ligand mixture, and the resulting solution was refluxed for 2 hours.

After cooling, the solid precipitate was filtered, washed with ethanol, dried, and weighed. The synthesis of the mixed ligand complex is illustrated in Scheme 1. The analytical and physical data of the synthesized complexes are presented in Table 1.



Scheme 1. Synthesis of Mixed ligand complex of P-MINAP and 1, 10-phenanthroline with Cu (II) metal ion.

Antimicrobial Activity

The antimicrobial activity of the synthesized compounds was evaluated using the agar well diffusion method [20]. The free Schiff base ligand (p-MINAP), 1,10-phenanthroline (1,10-Phen), and their mixed ligand metal complexes were tested in vitro for antibacterial and antifungal properties.

Antibacterial Activity:

The compounds were screened against Gram-positive bacteria (Bacillus cereus, Bacillus subtilis, Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa). Gentamycin and ampicillin were used as standard reference antibiotics for Gram-positive and Gram-negative bacterial strains, respectively.

Antifungal Activity:

Antifungal activity was assessed against Aspergillus niger, Fusarium oxysporum, and Candida albicans using Sabouraud Dextrose Agar (SDA) medium. Miconazole was employed as the standard antifungal agent.

All compounds were tested at a concentration of 0.5 mg/mL. Dimethyl sulfoxide (DMSO) was used as the solvent control. Sterile Petri dishes were prepared by pouring 20 mL of sterilized media into each dish and allowing them to solidify. Wells of 6 mm diameter were created using a sterile borer. Each plate was inoculated with a microbial suspension evenly distributed using a sterile swab. Test solutions were then introduced into the wells using a micropipette.

Plates were incubated at 35 °C for 24 hours for antibacterial activity, and at 25 °C for 48 hours for antifungal activity. All experiments were performed in triplicate, and the zones of inhibition were measured in millimeters to determine antimicrobial effectiveness.

III. RESULTS AND DISCUSSION

The elemental analysis results of the Schiff base ligand p-Methoxy Isonitrosoacetophenone (p-MINAP) and its mixed ligand complex were found to be in good agreement with the calculated values, confirming the proposed molecular formulae. Details such as elemental composition, molecular formulae, melting points, and yields of the synthesized compounds are summarized in Table 1.

The solubility of the mixed ligand complex in dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) enabled the determination of its molar conductivity in 2×10^{-4} M solution at 25 ± 2 °C. The measured conductivity values (listed in Table 1) indicate that the complex is ionic in nature. This ionic behavior suggests that the chloride anions are not coordinated to the metal center but are instead present in the outer coordination sphere, acting as counterions [21].

Table 1- Analytical and Physical data of the Novel Schiff base ligand and transition metal complex.

Compound	Colour	Molecular Weight	M. P. (°C)	% found (Calculated)				μ_{eff} (B.M)	Am (Ω^{-1} mol^{-1} cm^2)
				% C	%H	%N	%M		
p-MINAP	Brown	179.16	96	60.33 (60.54)	5.06 (5.64)	7.82 (7.98)	--	---	---
[Cu (p-MINAP) (1, 10 – Phen)]	Light green	422	183	59.64 (59.89)	4.05 (4.37)	9.93 (10.01)	15.02 (15.34)	1.78	143
[Ni (p-MINAP) (1,10-Phen)]	Light brown	418.06	188	60.27 (60.98)	3.86 (4.05)	10.05 (10.32)	14.04 (14.27)	2.82	135
[Co (p-MINAP) (1,10-Phen)]	Yellowish	418.30	189	60.24 (60.76)	3.85 (4.01)	10.04 (10.15)	14.08 (14.13)	3.87	132

IV. UV-VISIBLE SPECTRA

The UV-Visible absorption spectra of the Schiff base ligand p-MINAP and its mixed ligand complexes were recorded in DMF using 1 mM solutions over the wavelength range of 200–700 nm.

For p-MINAP, an absorption band was observed at 268 nm, which is attributed to the π–π* transition of the aromatic ring. A second band appeared at 308 nm, corresponding to the n–π* electronic transition of the azomethine (–CH=N–) group [16, 22].

In the spectra of the mixed ligand complexes of Cu(II), Ni(II), and Co(II), additional absorption bands were observed at 425 nm, 435 nm, and 455 nm, respectively. These are characteristic of d–d transitions, indicating the splitting of the d-orbitals in an octahedral field. Furthermore, all mixed ligand complexes exhibited a band around 332 nm, which

can be attributed to ligand-to-metal charge transfer (LMCT) transitions [21].

V. FT-IR SPECTRA

The FT-IR spectral data of the free ligands (p-MINAP and 1,10-phenanthroline) and their corresponding mixed ligand metal complexes are summarized in Table 2. In the spectrum of p-MINAP, the azomethine group exhibited a characteristic ν(C=N) stretching vibration at 1610 cm^{-1}, while 1,10-phenanthroline showed a ν(C=N) band (associated with the pyridyl nitrogen) at 1648 cm^{-1} [17].

In the spectra of the metal complexes, the ν(C=N) stretching frequency of the azomethine group was shifted to a higher wavenumber, indicating coordination of the azomethine nitrogen to the metal center. Similarly, a band observed at 1128 cm^{-1} in the spectrum of free 1,10-phenanthroline—assigned to aromatic ring (benzene and pyridine) stretching—also shifted to a higher frequency in the complexes, further supporting the involvement of nitrogen atoms in coordination.

The band at 654 cm^{-1} in free 1,10-phenanthroline, attributed to pyridyl nitrogen bending vibrations, was shifted to the range of 678–691 cm^{-1} in the complexes, again confirming metal coordination.

The ν(O–H) stretching vibration of the oxime group in free p-MINAP appeared at 3275 cm^{-1}, but was absent in the spectra of the complexes. This absence indicates deprotonation and coordination of the oxime group via the oxygen atom. Additionally, the strong ν(C=O) band at 1710 cm^{-1} observed in free p-MINAP disappeared in the complex spectra, suggesting involvement of the carbonyl group in coordination.

A band around 2840 cm^{-1} corresponding to the –OCH_3 group was observed and remained mostly unchanged. New bands appearing in the region of 508–543 cm^{-1} and 435–478 cm^{-1} in the complex spectra, which were absent in the free ligands, are attributed to ν(M–O) and ν(M–N) stretching vibrations, respectively, confirming coordination of both oxygen and nitrogen donor atoms to the metal center [23–24].

Table 2. FT-IR spectral data of P-MINAP, 1, 10-phene and Mixed ligand complexes with Cu(II), Ni(II) and Co(II) metal ions.

Probable Assessment	P-MINAP	1,10-Phen	[Cu(P-MINAP) (1,10-phen)]	[Ni(P-MINAP) (1,10-phen)]	[Co(P-MINAP) (1,10-phen)]
Aromatic CH	3018	3062	3038	3046	3011
-OH of =N-OH	3317	--	---	---	---
-OCH_3 stretch	2840	----	2841	2849	2820
-C=O stretch	1710	----	----	----	---
-C=N stretch	1610	1653	1637	1640	1651
=CH stretch	1440	1423	1449	1458	1445
=N→O	----	----	1235	1238	1233
Benzene & Pyridine ring stretching	---	1129	1142	1160	1154
Para substitution	766	---	769	772	765
M→O	----	-----	563w	524w	518w
M→N	---	-----	486s	464s	467s

^{1}H NMR Spectra

The ^{1}H NMR spectrum of the Schiff base ligand p-MINAP displayed a singlet at 2.42 ppm, corresponding to the azomethine (–CH=N–) proton. A set of multiplet signals was observed in the range of 7.79–8.29 ppm, attributed to aromatic protons, along with a singlet at 3.90 ppm assigned to the methoxy (–OCH_3) group.

Upon complexation, notable shifts in the chemical environment of various protons were observed. In the

spectra of the mixed ligand metal complexes, the azomethine proton signal was shifted downfield to the range of 2.45–2.49 ppm, indicating coordination of the azomethine nitrogen to the metal center. Additionally, the aromatic proton signals of 1,10-phenanthroline appeared further downfield, between 7.82–9.62 ppm, consistent with coordination of the nitrogen atoms of the phenanthroline ring to the metal ion [25].

These downfield shifts confirm the involvement of both the azomethine nitrogen of p-MINAP and the nitrogen atoms of 1,10-phenanthroline in metal coordination.

VI. BIOLOGICAL ACTIVITY

The Schiff base ligand (p-MINAP), 1,10-phenanthroline, and their corresponding metal complexes were evaluated in vitro for antimicrobial activity using the agar well diffusion method [20]. The compounds were tested against selected pathogenic bacterial and fungal strains to assess their antibacterial and antifungal efficacy.

Microorganisms Tested:

- Gram-positive bacteria: Staphylococcus aureus, Bacillus subtilis
- Gram-negative bacteria: Pseudomonas aeruginosa, Escherichia coli
- Fungi: Aspergillus niger, Candida albicans

Gentamicin, ampicillin, and miconazole were used as standard drugs for Gram-negative bacteria, Gram-positive bacteria, and fungi, respectively. The antimicrobial activity of the compounds was assessed by measuring the diameter of the inhibition zones, and the results are summarized in Table 3.

Antibacterial Activity:

The free Schiff base ligand (p-MINAP) exhibited moderate antibacterial activity against all tested bacterial strains, although its activity was lower than that of the standard antibiotics. The mixed ligand complexes demonstrated significantly enhanced antibacterial activity compared to the free ligand and 1,10-phenanthroline alone.

Among the metal complexes, the Co(II) complex showed the highest antibacterial activity against both Gram-positive and Gram-negative bacteria. This enhanced activity may be attributed to the chelation theory [26], which suggests that coordination of the ligands with metal ions increases the lipophilicity of the complexes, thereby facilitating their penetration through the bacterial cell membrane and enhancing their biological activity.

Antifungal Activity:

The Ni(II) complex exhibited the most potent antifungal activity against Aspergillus niger, outperforming all other compounds and even the standard antifungal drug. In contrast, the Co(II) complex showed relatively low antifungal activity against Candida albicans when compared to the other complexes.

The antifungal activity data are also presented in Table 3, highlighting the variable efficacy of the complexes depending on the metal center and the target organism.

Table 3- Antibacterial and Antifungal activity of Novel Schiff's base ligand and transition metal complexes

Sr. No.	Compound	Inhibition Zone diameter (mm/mg)					
		Gram +ve bacteria		Gram –ve bacteria		fungi	
		S. aureus	B. subtilis	P. aeruginosa	E. coli	A. niger	C. albican
1	p-MINAP	+++	++++	+++	+++	+++	+++
2	1, 10- phen	+++	+++	++	+++	NA	NA
3	[Cu(p-MINAP)(1,10-phen)]	++++	++++	++	++	+++	+++
4	[Ni(p-MINAP)(1,10-phen)]	++++	++++	+++	+++	++++	++++

5	[Co(p-MINAP)(1,10-phen)]	++++	++++	+++	++++	++++	+++
6	Ampiciline	++++	++++	NA	NA	NA	NA
7	Gentamycine	NA	NA	++++	++++	NA	NA
8	Miconazole	NA	NA	NA	NA	++++	++++

Note: NA: No activity, Inhibition values: 1–5 mm beyond control = +, 6–10 mm beyond control = ++, 11–15 mm beyond control = +++, >15 mm beyond control = ++++.

VII. CONCLUSION

A new Schiff base ligand (p-MINAP), derived from p-methoxyacetophenone and n-amyl nitrite, was synthesized and used as a primary ligand in the formation of mixed ligand complexes with transition metal ions, alongside 1,10-phenanthroline (1,10-phen) as the secondary ligand.

The free ligand and its mixed ligand complexes with Cu(II), Ni(II), and Co(II) were characterized using elemental analysis, IR spectroscopy, UV-Vis spectroscopy, magnetic susceptibility, and ^{1}H NMR spectroscopy. Spectral data suggested that the metal complexes adopt an octahedral geometry. Molar conductivity measurements indicated that the complexes are electrolytic in nature.

Thermal analysis further supported the proposed molecular formulae and confirmed the thermal stability of both the Schiff base ligand and its metal complexes.

The synthesized compounds were also evaluated for their antimicrobial activity. The mixed ligand complexes exhibited significant antibacterial and antifungal activity compared to the free ligands, demonstrating promising biological potential. These findings highlight the relevance of such Schiff base complexes in coordination chemistry and bioinorganic applications.

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