



**SYNTHESIS AND BIOLOGICAL EVALUATION OF MIXED LIGAND
COMPLEXES DERIVED FROM AZO DYE AND 2-AMINO-4-NITROPHENOL
USING TRANSITION METAL IONS**

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ABSTRACT

Present communication deals with synthesis, characterization and antimicrobial screening of mixed ligand complexes derived from 2,4-dimethyl-3-aryazo-6-thiopyrimidine and 2-amino-4-nitrophenol using transition metal ions viz. Mn(II), Co(II), Ni(II), Cu(II) and Zn(II). The characterization of these synthesized complexes has been carried out with the help of spectral techniques such as IR, ¹H-NMR and electronic spectra. The antimicrobial activities carried out on the derived complexes reveal that all these complexes are biologically active against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas diminuta*.

KEYWORDS:

2-Amino-4-Nitrophenol; 2,4-dimethyl-3-aryazo-6-thiopyrimidine; Mixed ligand complexes; Antimicrobial activity.

INTRODUCTION

Mixed ligand complexes are known to possess at least two different kinds of ligands associated with the same metal ion and they differ from traditional metal complexes. The complex may vary in expected properties due to existence of more than one type of ligand. Due to this uniqueness, such mixed ligand complexes attracts the researchers to involve in this field. A variety of reports have accounted for the synthesis and characterization of mixed ligand complexes [1-6]. The coordination of ligand to metal center changes the electronic property and the ligand itself may undergo reaction with nucleophiles at an improved rate as compared to that of free ligands. This is yet a growing field in the metal assisted organic transformation [7, 8].

Aryl azoheterocycles are potential acidic ligands and undergo varieties of metal assisted organic transformation which are otherwise impossible [9-13].

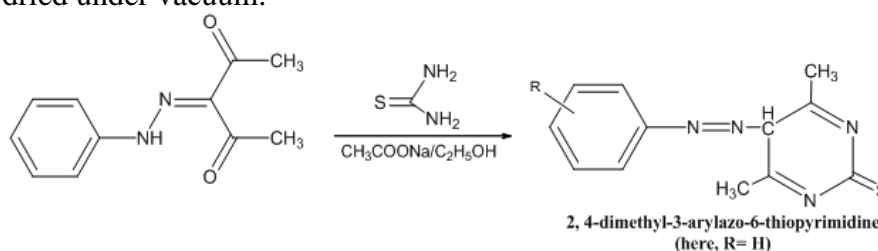
In the present work, we report the synthesis and characterization of five mixed ligand complexes derived from 2,4-dimethyl-3-aryazo-6-thiopyrimidine and 2-amino-4-nitrophenol using transition metal ions Mn(II), Co(II), Ni(II), Cu(II) and Zn(II); which is an extension to

the similar work done earlier in our lab [13-15]. The synthesized complexes were screened for their antimicrobial activities.

EXPERIMENTAL: All the reagents (from Sigma/Merck) were used as such. IR spectra were recorded on a Perkin Elmer spectrophotometer (Spectrum Version 10.4.00) in the 4000-400 cm^{-1} range using KBr pellets. $^1\text{H-NMR}$ spectra were recorded in $\text{d}_6\text{-DMSO}$ using TMS as an internal reference on a Bruker Ascend 300 MHz system. UV-visible wavelengths were calculated on SL-159 Single Beam Microprocessor based Scanning UV-Visible Spectrophotometer. Melting points were observed in an open capillary tube using an electric melting point apparatus and are uncorrected. The IR & $^1\text{H-NMR}$ studies were carried out at MNIT, Jaipur. UV-visible data and anti-microbial activity were carried out at Biotech Dept., School of Engineering & Technology, Jaipur National University, Jaipur.

Synthesis of 2, 4-dimethyl-3-arylozo-6-thiopyrimidine [21]

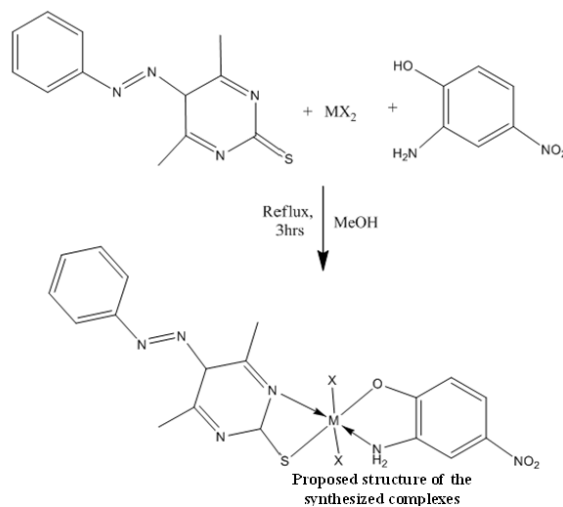
3-(2-phenylhydrazono)pentane-2,4-dione (4.71 g, 23.078 mmol) and thiourea (1.75 g, 23.078 mmol) were mixed together in the presence of freshly prepared sodium ethoxide solution (1.6 g sodium metal in 50 ml absolute ethanol). This solution was stirred for 3–4 h at room temperature, eluted over silica gel and solvent was stripped off to obtain good yield (75-80%) crystals of 2, 4-dimethyl-3-arylozo-6-thiopyrimidine (Scheme 1). The crystals were then filtered and dried under vacuum.



Scheme 1. Synthesis of 2, 4-dimethyl-3-arylozo-6-thiopyrimidine

Synthesis of mixed ligand complexes [22]

A hot methanolic solution of zinc chloride (1.6 g, 11.74 mmol) was mixed with an equimolar quantity of 2,4-dimethyl-3-arylozo-6-thiopyrimidine (2.87 g) and 2-amino-4-nitrophenol (1.81 g). Few drops of conc. HCl were added to this mixture and the contents were allowed to reflux for 4 hours (Scheme 2). Resultant complexes were precipitated out on cooling, filtered, washed with methanol and recrystallized from ethanol to obtain $[\text{Zn}(\text{C}_{18}\text{H}_{18}\text{N}_6\text{O}_3\text{S})\text{Cl}_2]$.



Scheme 2. Synthesis of Mixed ligand Complexes

The other complexes were synthesized with similar procedure. The synthesized complexes were obtained in 60-70% yield.

RESULT AND DISCUSSION

General composition of complexes

The general composition of the synthesized mixed ligand complexes can be represented as $[M(C_{18}H_{18}N_6O_3S)Cl_2]$ (where M = Mn, Co, Ni, Cu & Zn). All the synthesized complexes are found to be thermally stable and coloured. Physical parameters were recorded and are given in Table 1.

Table 1

Physicochemical parameters of synthesized mixed ligand complexes

S. No.	Complex	Color	Melting points (°C)	Yield (%)
1	$[Zn(C_{18}H_{18}N_6O_3S)Cl_2]$	Dark Brown	200	68
2	$[Cu(C_{18}H_{18}N_6O_3S)Cl_2]$	Dark Blue	220	81
3	$[Mn(C_{18}H_{18}N_6O_3S)Cl_2]$	Black	205	76
4	$[Co(C_{18}H_{18}N_6O_3S)Cl_2]$	Dark Green	214	79
5	$[Ni(C_{18}H_{18}N_6O_3S)Cl_2]$	Light Brown	219	79

IR Spectra

IR spectra of all the synthesized complexes have been recorded in the range 4000-400 cm^{-1} . The spectra are interpreted considering few main peaks observed. The presence of peaks around 3400 cm^{-1} account for the presence of $\nu(-NH_2)$ group in the complexes.

The bands observed around 1445-1450 cm^{-1} and 2990-3055 cm^{-1} have been assigned to $\nu(C=C)$ and $\nu(C-H)$ aromatic stretching, respectively. The peaks observed in the region around 1600 cm^{-1} for all the complexes is due to presence of $\nu(>C=O)$ group in complexes. The peaks observed around 1525 cm^{-1} can be assigned due to $\nu(-N=N-)$ group present in the complexes [16].

The weak intensity peaks observed in the region 505-645 cm^{-1} can be attributed to $\nu(Metal-O)$ and $\nu(Metal-N)$ present in the complexes. The $\nu(Metal-Cl)$ band is usually observed below 400 cm^{-1} region.

1H -NMR Spectra

NMR spectrum of 2, 4-dimethyl-3-arylo-6-thiopyrimidine exhibit a singlet at δ 1.41 ppm due to the presence of isopropenyl methyl groups (Table 2). Another singlet at δ 2.33 ppm has been assigned to two methyl entities of thiopyrimidine ring. The peak at δ 6.91 ppm can be attributed to methine proton. A small hump at δ 14.11 ppm accounts for the tautomeric behavior of proton on nitrogen of imidazole ring, with methine proton. Doublet at δ 7.58 ppm can be assigned to the H_a and H_d protons due to ortho coupling of the order of $J = 8.0$ Hz. Similarly, a triplet at δ 7.08 ppm is associated with H_b , H_c aromatic protons with $J = 8.0$ Hz, characteristic of ortho-coupling with neighboring protons. A blurred singlet at δ 8.35 ppm shows the presence of $(-C-H)$ proton present in the pyrimidine ring. The well resolved multiplet at δ 7.2-7.7 ppm corresponds to the aromatic protons in mixed ligand complexes and the singlet broad peak observed at δ 4.5 ppm is assignable to $(-N-H)$ protons.

Table 2¹H-NMR spectral data for synthesized mixed ligand complexes

S. No.	Complex	¹ H-NMR
1	[Zn(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	1.4 (s, 6H, 2 CH ₃); 1.8 (s, CH, ring); 7.1 (t, Ar-H); 7.2 (d, Ar-H); 7.5 (d, Ar-H), 8.0 (s, aromatic N-H)
2	[Cu(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	1.5 (s, 6H, 2 CH ₃); 1.71(s, CH, ring); 7.05 (t, H-4, H-5, Ar-H); 7.2 (d, H-6, Ar-H); 7.5 (d, H-3, Ar-H); 8.1 (s, aromatic N-H); 8.15 (m, 6H-benzene)
3	[Mn(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	1.5 (s, 6H, 2 CH ₃); 1.70 (s, CH, ring); 7.10 (t, H-4, H-5, Ar-H); 7.4 (d, H-6, Ar-H); 7.2 (d, H-3, Ar-H); 8.0 (s, aromatic N-H); 8.18 (m, 6H-benzene)
4	[Co(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	1.5 (s, 6H, 2 CH ₃); 1.71 (s, CH, ring); 7.4 (t, H-4, H-5, Ar-H); 7.6 (d, H-6, Ar-H); 7.8 (d, H-3, Ar-H); 8.5 (s, aromatic N-H); 8.41 (m, 6H-benzene)
5	[Ni(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	1.5 (s, 6H, 2 CH ₃); 1.71 (s, CH, ring); 7.05 (t, H-4, H-5, Ar-H); 7.2 (d, H-6, Ar-H); 7.5 (d, H-3, Ar-H); 8.1 (s, aromatic N-H); 8.15(m, 6H-benzene)

UV Spectroscopy

The UV-vis spectroscopy for all the synthesized complexes was recorded in DMSO. Azo compounds usually show two to three bands are reported for azo compounds [17-19]; the band around 200-220 nm is assignable to $\pi \rightarrow \pi^*$ of the benzenoid moiety and the band around 420-425 nm can be accounted for $n \rightarrow \pi^*$ electronic transition of -N=N- group. Shift in positions of these bands signifies that the azo compounds form a complex with metal atom through -N=N- group. In present case, these two bands are not significantly altered and are almost undisturbed suggesting that the -N=N- group is not taking part in bond formation with the metal ion. Apart from these, specific bands assigned to metal ligand interaction were also observed in these complexes (Table 3).

Table 3

UV-vis spectral data of synthesized complexes

S. No.	Metal Complex	Wavelength (nm)
1	[Zn(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	650 nm
2	[Cu(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	580 nm
3	[Mn(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	670 nm
4	[Co(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	685 nm
5	[Ni(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	570 nm

Antimicrobial Activity

The in vitro antibacterial activity of the mixed ligand complexes have been investigated against several pathogenic bacteria. The Minimal inhibitory concentration (MIC) was determined by means of Kirby-Bauer well diffusion method using nutrient agar media [20]. The bacterial suspension prepared in peptone water compared to 0.5 Mc Farland Turbidity standards. Bacterial plates were incubated at 37°C for 24 h. Standard laboratory cultures of four microbial strains i.e. *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas diminuta* were used in antimicrobial assay. Two concentrations of the test compounds of the stock solution (1X and 0.5X) were prepared for the compound and 100 μ l of each concentration

was used in separate wells. Streptomycin was used as positive control and ethyl alcohol as negative control for antimicrobial activity. The screening results (Table 4, Figure 1) reveal a remarkable effect of metal complexes on the bacteriocidal/ bacterostatic potency, as per the pattern:

S. aureus>*B. subtilis*>*P. diminuta*>*E. coli*

Table 4: Screening resultsof mixed ligand complexes

S. No.	Complex	-log MIC (in µg/ml)			
		SA	BS	PD	EC
1	[Zn(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	4.825	4.177	4.625	4.579
2	[Cu(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	4.665	4.624	4.551	4.519
3	[Mn(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	4.402	4.380	4.338	4.319
4	[Co(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	4.752	4.585	4.471	4.301
5	[Ni(C ₁₈ H ₁₈ N ₆ O ₃ S)Cl ₂]	4.645	4.495	4.377	4.269

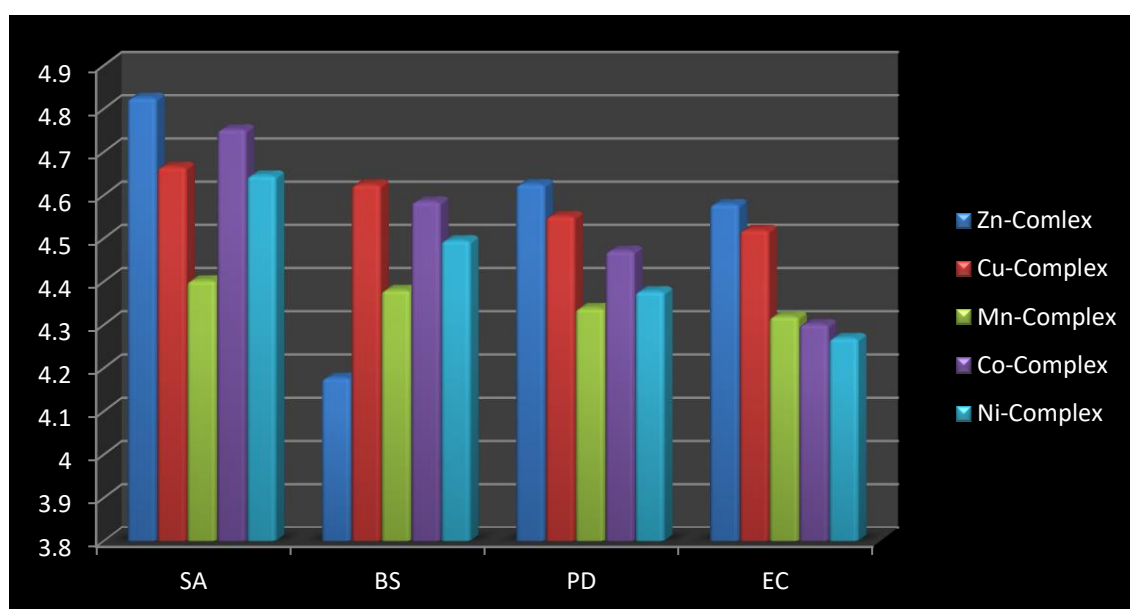


Figure 1. Anti-microbial screening resultsof mixed ligand complexes (Y-axis accounts for -log MIC values in µg/ml)

CONCLUSION

In this work, we have reported mixed ligand complexes synthesized by reacting 3-(2-phenylhydrazono)pentane-2,4-dione and 2-Amino-4-nitrophenol with respective metal chlorides. These synthesized complexes have been characterized with the help of spectral techniques such as IR, electronic spectra and a six coordinated octahedral geometry can be proposed for all these complexes. The anti-microbial of all these complexes reveal their active biological significance.

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