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# Synthesis, Spectral Analysis of Azo-Dye Ligands and Complexes and there Antimicrobial Studies

**Kuruba Siddappa* and Harshavardhan Sagar**
*Department of Chemistry, Gulbarga University, Kalaburagi 585 106, India*

**Abstract:**
Several azo-dyes schiff bases have been synthesized by reaction of salicylaldehyde containing azo dyes with various substituted aniline derivatives in the presence of catalytic amount of acetic acid. The structures of diazenyl derivatives were determined by FTIR, UV–Vis, $^{1}$H NMR, $^{13}$C NMR, and mass spectroscopic studies. The synthesized derivatives were screened for their *in-vitro* antibacterial activity against three gram-positive bacterium, *Enterococcus fecalis (E.fecalis), Staphylococcus aureus (S.aureus) and Bacillus subtilis (B. Subtilis)* and two gram-negative bacteria *Klebsiella pneumonia(K. pneumoniae)and Pseudomonas aeruginosa (P. aeruginos)* strains, using streptomycin as standard drugs. The schiff bases exhibited significant activity toward both Gram-positive, Gram-negative bacterial strains.

**Keywords:** Schiff base, azo dye, salicylaldehyde, antimicrobial, complexes, and spectral studies.

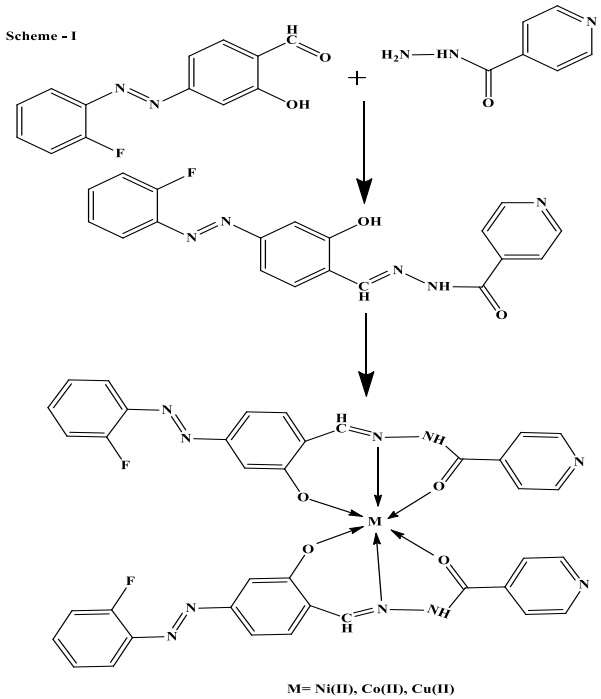
## INTRODUCTION

An azo coupling is an organic reaction normally proceeds between a diazonium compound and other aromatic compound that produces an azo compound (-N=N-). Azo compounds possess the importance in the synthesis of active antibiotics like sulfonamides and many other potent organic molecules used in day-to-day medications. Azo derivatives[1] are a class of privileged chemical compounds, continuously receiving attention in cosmetic and pharmaceutical research [2] They have become unavoidable tools for a wide range of industries like dyes, plastics, textiles, food processing and biotechnology industries, life without dyes can't be imagined.

Azo Schiff base metal complexes have been studied extensively for years due to the synthetic flexibilities of these Schiff base ligands and their selectivity as well as sensitivity towards the transition metal ions [3]. Among the ligand systems, hydrazide and hydrazones occupy special place because transition metal complexes of these ligands developed due to their chelating capability, structural flexibility, interesting electrical as well as magnetic properties [4], and, nowadays, they are extensively being used for their promising applications in the treatment of several diseases and also been used as synthetic and analytical reagents [5]. Moreover, these ligands exhibit keto-enol tautomerism [6-8] bearing unusual coordination numbers. In the present paper, we report the synthesis and characterization of Co(II), Ni(II), Cu(II), complexes of azo Schiff base derived from the condensation of.

Azo compounds or dyes characterized by the presence of the azo moiety (–N=N–) in their structure, conjugated with two, distinct or identical, mono- or polycyclic aromatic or hetero aromatic systems (9). Previous studies have shown the antimicrobial activity of metal complexes against various pathogens including bacteria (10). However, the antimicrobial activity of any synthetic metal complexes might be different and therefore, it should be revealed.

**Scheme:**




## RESULT AND DISCUSSION

### Chemistry

We report here in, a simple and expeditious synthetic rout for the synthesis of various derivatives of azo dyes with INH as a base. Synthetic pathway for the synthesis of indole derivatives is summarized in (Scheme). In a typical procedure, a mixture of INH and (E)-N'-(4-((E)-(2-fluorophenyl)diazenyl)-2-hydroxybenzylidene) isonicotinohydrazide (0.01mol) containing 4-5 drops of glacial acetic acid was refluxed in methanol (35mL) on a water bath for 6hrs. The reaction contents were cooled and poured into ice cold water. The resulting solid was filtered, washed with sodium bisulphate solution followed by cold water, dried and recrystalized from ethanol to get pure compounds **(L1)**. The final

compounds **(HSL1, HTL1, and THL1)** were prepared by adding 10 mL of an ethanolic metal salt (4 mmol) to a ethanol/chloroform (1:1 v/v) with **L1** and the mixture was refluxed for 6 hr. The obtained solution was left at room temperature and the resulting precipitates were filtered off, washed with ethanol and then recrystallized from an ethanol/ chloroform (1:3 v/v) solvent mixture. The newly synthesized compounds were screened for *in-vitro* antibacterial activity against three gram-positive bacterium, *Enterococcus fecalis (E.fecalis), Staphylococcus aureus (S.aureus) and Bacillus subtilis (B. Subtilis)* and two gram-negative bacteria *Klebsiella pneumonia(K. pneumoniae) Pseudomonas aeruginosa (P. aeruginosa)*.

**Biological Evaluation**

**In-vitro antimicrobial activity**

The investigation of antimicrobial screening revealed that, test compounds showed varying degrees of activity against all the tested microorganisms to quantify the antimicrobial potency of the compounds and the results have been given in (Table). It is clear from our present findings that heterocyclic systems with electron withdrawing F groups on the phenyl ring play an important role in varying the efficacy of antimicrobial activity. The role of electron with drawing group in improving antimicrobial activities had been reported in the literature [14]. The investigation of the tested compounds revealed that the series of compounds tested was found to be the most active when compared with the standards *Streptomycin*.

## EXPERIMENTAL

**Chemistry**

All chemicals used in this research were purchased from sigma Aldrich and SD Fine chemicals. Melting points were taken in open capillary tubes and are uncorrected. The purity of the compounds was checked by thin layer chromatography using Merck silica gel 60 F254 coated aluminum plates. IR spectra were recorded on Shimadzu FT-IR Infrared spectrometer in KBr pellets(100mg). $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker (400 and 100MHz) tetramethylsilane (TMS) as an internal standard. LCMS were obtained using Agilent 1200 series Mass spectrometer (SAIF) Punjab.

**Protocol for the synthesis of starting materials**

**Isonicotinohydrazide (INH):** INH was purchased from sigma Aldrich chemicals.

**Synthesis of Ligand (L1):**

To a solution of 2-Floroaniline (10 mmol) in water (5 mL), concentrated hydrochloric acid (20 mL) was added slowly with stirring. The clear solution was poured into ice water mixture, diazotized with sodium nitrite (0.69 g, 10 mmol), dissolved in water (3.5 mL), during a period of 15 min at 0-5ºC. The cold diazo solution was added drop wise to the solution of salicyladehyde (1.05 mL, 10 mmol) in water (50 mL) containing sodium hydroxide (0.4 g) and sodium carbonate (7.3 g) during a period of 30 min at 0-5ºC. The reaction mixture was stirred for 1 hr in ice bath, allowed to warm slowly to room temperature and subsequently stirred for 4 hr at this temperature. The product was collected by filtration and recrystallized from mixture of EtOH and $H_2O$.

**Synthesis of Schiff bases: (E)-N'-(4-((E)-(2-fluorophenyl)diazenyl)-2-hydroxybenzylidene) isonicotinohydrazide:**

Azo dyes (1mmol) in MeOH (7mL) were added to a solution of isonicotinohydrazide (INH) (1 mmol, 0.11 g) in MeOH (7mL) containing 4-5 drops of glacial acetic acid was refluxed in methanol (35mL) on a water bath for 6hrs. The reaction contents were cooled and poured into ice-cold water. The resulting solid was filtered, washed with sodium bisulphate solution followed by cold water, dried and recrystalized from ethanol to get pure compounds.

**Syntheses of metal complexes**

Ni (II), Co (II) and Cu (II) complexes were prepared by adding 10 mL of an ethanolic metal salt (4 mmol) to a ethanol/chloroform (1:1 v/v) solution containing 8 mmol of the ligand (L1) and the mixture was refluxed for 6 hr. The obtained solution was left at room temperature and the resulting precipitates were filtered off, washed with ethanol and then recrystallized from an ethanol/ chloroform (1:3 v/v) solvent mixture.

**Biological Evaluation**

**Antimicrobial activity**

The newly synthesized compounds were screened for *in-vitro* antibacterial activity against three gram-positive bacterium, *Enterococcus fecalis (E.fecalis), Staphylococcus aureus (S.aureus) and Bacillus subtilis (B. Subtilis)* and two gram-negative bacteria *Klebsiella pneumonia(K. pneumoniae) Pseudomonas aeruginosa (P. aeruginosa)*. As reported [14].

For antibacterial, preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. Each test compound (5mg) was dissolved in 5ml of dimethyl sulfoxide (1000µg/ml).Volumes of 0.05ml and 0.1ml of each compound was used for testing. The cups each of 9mm diameter were made by scooping out medium with a sterilized cork borer in a petri dish which was streaked with the organisms. The solutions of each test compound (0.05 and 0.1ml) were added separately in the cups and petri dishes were subsequently incubated. *Streptomycin* used as standard reference drugs (200µg/ml) and dimethyl sulphoxide as a control which did not reveal any inhibition. Inoculated plates were kept at RT for 48hr. Each plate was then observed for zone of inhibition. Zone of inhibition produced by each compound was measured in mm.

*In-vitro* Antimicrobial activity.

| Compounds | Conc. (µg/ml) | MIC value | | | | |
|---|---|---|---|---|---|---|
| | | Antibacterial activities | | | | |
| | | *B. Subtilis* | E. *fecalis* | S. *aureus* | *K. pneumoniae* | *P. aeruginosa* |
| **L1** | **100** | **---** | **08** | **11** | **05** | **01** |
| | **0.75** | **---** | **13** | **---** | **01** | **02** |
| | **0.50** | **05** | **04** | **05** | **---** | **01** |
| | **0.25** | **05** | **08** | **11** | **---** | **---** |
| **HSL1** | **100** | **---** | **04** | **---** | **05** | **01** |
| | **0.75** | **---** | **06** | **---** | **03** | **01** |
| | **0.50** | **05** | **05** | **---** | **---** | **01** |
| | **0.25** | **02** | **06** | **01** | **---** | **01** |
| **HTL1** | **100** | **05** | **04** | **02** | **05** | **01** |
| | **0.75** | **---** | **05** | **---** | **---** | **05** |
| | **0.50** | **05** | **04** | **05** | **---** | **02** |
| | **0.25** | **---** | **03** | **02** | **---** | **03** |
| **THL1** | **100** | **---** | **02** | **05** | **03** | **---** |
| | **0.75** | **02** | **02** | **05** | **02** | **01** |
| | **0.50** | **---** | **04** | **---** | **04** | **---** |
| | **0.25** | **01** | **03** | **---** | **05** | **01** |
| **Std I** | **100** | **15** | **15** | **15** | **15** | **15** |
| | **0.75** | **10** | **10** | **10** | **10** | **10** |
| | **0.50** | **10** | **10** | **10** | **10** | **10** |
| | **0.25** | **08** | **08** | **08** | **08** | **08** |

Key for interpretation: '---'Not shown any zone of inhibition; Inactive: <1 mm, weakly active: 2-4 mm, moderately active: 4-6 mm, highly active: more than 6mm, Std I; Streptomycin.

**Spectral analysis of synthesized compounds**

**(E)-4-((2-fluorophenyl)diazenyl)-2-hydroxybenzaldehyde (L1).** Yellow shining crystals, yield 73%, m.p. 238-240ºC; IR(KBr, $cm^{-1}$); 3449 (OH), 2925(Ar-H), 1624(C=O), 1551 (C=C), 1486(N=N), 1165(-O-), 1099 $cm^{-1}$ (C-F). $^1$H NMR (400 MHz, Bruker δ ppm)(DMSO-$d^6$); 7.13 - 8.30 (m, 11H, Ar-H), 8.79 (s, 1H, NH), 11.70(s, 1H, OH) and 12.36 (s, 1H, CH=N). $^{13}$C NMR (100 MHz, Bruker δ ppm) (DMSO-$d^6$): 117, 119, 121, 123, 124, 126, 132, 139, 145, 146, 150(Ar-C), 157(CH=N) and 161.50(C=O). Mass; MS ($C_{19}H_{14}FN_5O_2$) (m/z) = 363.11($M^+$) (100%), 364.12 (M+2) (33%).

Chemical Formula:

**Metal Complex (HSL1 Ni)**. Light grayish powder, yield 72%, m.p. 360ºC; IR (KBr, $cm^{-1}$); 3411(M-O-C), 1606(CH=N), 1482(C=O), 1357(-O-) and 753 $cm^{-1}$(C-F). $^1$H NMR (400 MHz, Bruker δ ppm) (DMSO-$d^6$); 7.19 - 8.34 (m, 12H, Ar-H), 9.13 (s, 2H, NH) and 11.79 (s, 2H, CH=N).. $^{13}$C NMR (100 MHz, Bruker δ ppm) (DMSO-$d^6$): 115, 121, 123, 124, 126, 131, 138, 143, 144, (Ar-C) and 158.99(C=O). Mass; MS ($C_{56}H_{36}N_6O_4Ni$) (m/z) =782 ($M^+$).

**Metal Complex (HTL1 Co)**. Grayish powder, yield 62%, m.p. 360ºC; IR (KBr, $cm^{-1}$); 3437(M-O-C), 1608(CH=N), 1549(C=O), 1483(-O-) and 753 $cm^{-1}$(C-F). $^1$H NMR (400 MHz, Bruker δ ppm) (DMSO-$d^6$); 7.09 - 8.72 (m, 12H, Ar-H), 11.63 (s, 2H, NH) and 12.15 (s, 2H, CH=N). $^{13}$C NMR (100 MHz, Bruker δ ppm) (DMSO-$d^6$): 115, 121, 123, 124, 126, 131, 138, 143, 144, (Ar-C) and 158.99(C=O). Mass; MS ($C_{56}H_{36}N_6O_4Co$) (m/z) =782 ($M^+$).

**Metal Complex (THL1 Cu)**. Grayish powder, yield 78%, m.p. 264ºC; IR (KBr, $cm^{-1}$); 3435(M-O-C), 3225(NH), 3072(Ar-H), 2851(C-H), 1675(CH=N), 1608(C=O), 1592(-O-) and 783 $cm^{-1}$(C-F). $^1$H NMR (400 MHz, Bruker δ ppm) (DMSO-$d^6$); 7.11-8.932 (m, 12H, Ar-H), 10.36 (s, 2H, NH) and 11.09 (s, 2H, CH=N). $^{13}$C NMR (100 MHz, Bruker δ ppm) (DMSO-$d^6$): 115, 121, 123, 124, 126, 131, 138, 143, 144, (Ar-C) and 158.99(C=O). Mass; MS ($C_{56}H_{36}N_6O_4Cu$) (m/z) =787 ($M^+$).

## CONCLUSION

In conclusion, we have designed an efficient synthetic route for the synthesis of some novel biologically active molecules. This observation suggests that disubstitution in the target compounds by halogens (F) enhanced the antimicrobial activities.

The coordination ability of the newly synthesized azo Schiff base has been proved in complexation reaction with Co(II), Ni(II) and Cu(II) ions. IR, UV-vis spectra, of ligand and its metal complexes confirmed the suggested coordination of the ligand through phenolic carbonyl oxygen, oxygen of OH group, and nitrogen of

the azomethine group as tridentate. The process of chelation dominantly affects the biological activity of the complexes that are potent against pathogens. In general, all the synthesized compounds can serve as potential microbial agents. Based on these facts, it could be proposed that these novel materials can be better accommodated
for biological applications.

**Competing interests**

The authors declare that they have no competing interests.

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