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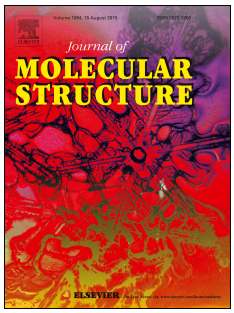

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N.M. Mallikarjuna, J. Keshavayya, M.R. Maliyappa, R.A. Shoukat Ali, Talwara Venkatesh

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Synthesis, Characterization, thermal and biological evaluation of Cu (II), Co (II) and Ni (II) complexes of azo dye ligand containing Sulfamethaxazole moiety

Mallikarjuna N M[a], Keshavayya J[a]*, Maliyappa M R[a], Shoukat Ali R A[a], Venkatesh Talwara[a]

[a]Department of PG Studies and Research in Chemistry, School of Chemical Sciences, Kuvempu University, Jnana Sahyadri, Shankaraghatta-577451, Karnataka, India

[*]E-mail: jkeshavayya@gmail.com

Abstract:

A novel bioactive Cu (II), Co (II) and Ni (II) complexes of the azo dye ligand (**L**) derived from sulfamethoxazole were synthesized. The structures of the newly prepared compounds were characterized by elemental analysis, molar conductance, magnetic susceptibility, FTIR, UV- visible, ^{1}H NMR, mass, thermal and powder XRD spectral techniques. Molar conductivity measurements in DMSO solution confirmed the non-electrolytic nature of the complexes. All the synthesized metal complexes were found to be monomeric and showed square planar geometry except the Co (II) complex which has six coordinate, octahedral environment. The metal complexes have exhibited potential growth inhibitory effect against tested bacterial strains as compared to the free ligand. The ligand and complexes have also shown significant antioxidant and Calf Thymus DNA cleavage activities. Further, the *in silico* molecular docking studies were performed to predict the possible binding sites of the ligand (**L**) and its metal complexes with target receptor Glu-6P.

Keywords: Sulfamethoxazole, azo dyes, mononuclear complexes, TG-DTA, antimicrobial.

1. Introduction:

Over the last four decades, azo dyes containing heterocyclic ring have played a vital role in the development of coordination chemistry [**1**]. The utility of these compounds is due to their versatile applications in the field of biological, electrochemical and analytical investigations [**2, 3**]. The metal complexes of heterocyclic ligands containing S and N atoms have been extensively studied due to their potential cytotoxicity, antimicrobial, anti-mycobacterial as well as antioxidant activities [**4-5**]. Significant efforts have been made to carry out studies on synthesis and biological activities of different metal (II) complexes of azo dyes [**6**]. Therefore, the heterocyclic azo dyes have proved to be efficient chelating agents for various metal ions, which is due to the presence of azo group responsible for exhibiting excellent medicinal properties. Further, azo metal complexes have been found to be effective in designing thermally stable optical storage devices and anticorrosive agents for metals [**7, 8**].

The presence of sulfonamide unit in the coordination complexes exhibit excellent pharmacological properties and has been used as effective drugs for the treatment of various diseases [**9**]. Among the sulfonamides, sulfamethoxazole continued to be utilized as an active chemotherapeutic agent as well as for the prevention of bacterial infection. Even though the sulfonamides are being widely used for the treatment of several diseases, their utility is limited due to toxicity and side effects that cause respiratory problems, kidney damage, and headache in case of frequent use [**10, 11**]. Due to these impending challenges, researchers are focusing on the development of metal based-drugs which have shown enhanced pharmacological and toxicological properties [**12**]. Similarly, recent studies showed that sulfonamide metal chelates had been found to be potential biological agents [**13, 14**]. Theoretical approaches have become more significant as efficient facilitating techniques in the development of novel drugs. Molecular docking is one such a tool which helps to find the

possible binding sites of the compounds against its biological receptor [**15**]. Further, it provides a platform to understand the mechanism of drug action and thereby we can design efficient drugs to cure the particular diseases [**16, 17**].

In the present study, we have synthesized novel bioactive Cu (II), Co (II) and Ni (II) complexes of the azo dye ligand (**L**) derived from sulfamethoxazole and characterized by various analytical and spectroscopic techniques. The electronic, magnetic and thermal techniques were used to deduce the geometry of the metal complexes. Further, the biological significance of the ligand and its metal complexes was investigated.

2. Experimental:

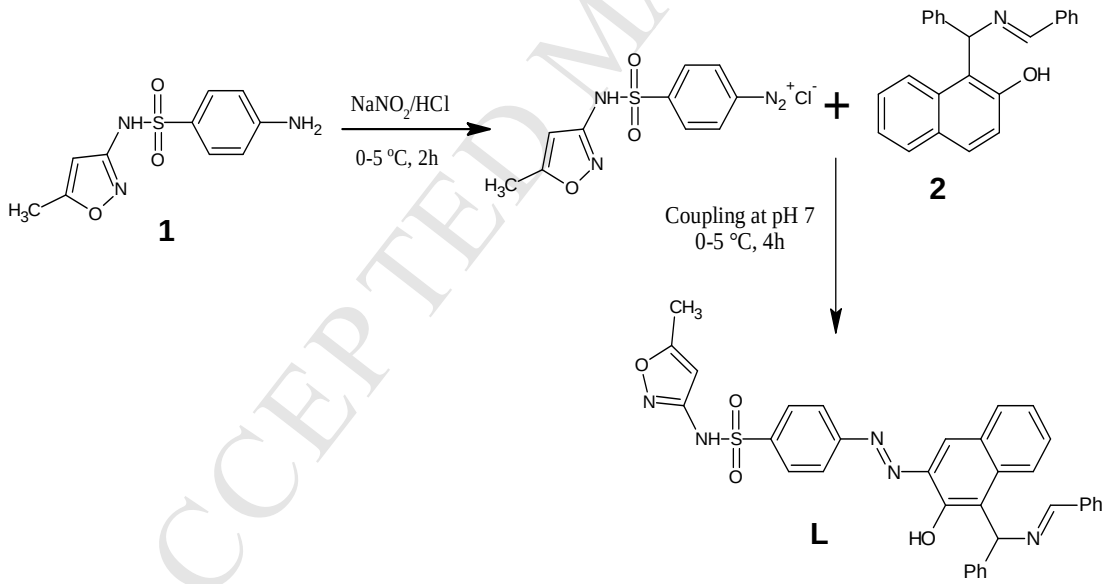
2.1 Chemicals and methods

All chemicals and solvents used for the synthesis were AR grade and purchased from Sigma Aldrich Chemical Company and used without further purification. Melting point was checked by electro-thermal apparatus using open capillary tubes and are uncorrected. Elemental analysis was obtained from Vario EL III CHN analyzer, and metal analysis was carried out by the following standard methods [**18**]. The UV-Visible spectra of the newly synthesized compounds were recorded on Elico-SL 164 double beam spectrometer in the range of 200-800 nm in DMSO solution (10^{-6} M). IR spectra were recorded on a Perkin Elmer- Spectrum RX-IFTIR instrument as KBr pellets in the region of 4000-200 cm^{-1}. ^{1}H NMR spectrum of the ligand was recorded in d_6- DMSO using an Avance III instrument. LCMS spectra were recorded on LCMS 2010, SHIMADZU mass analyser. Molar conductivity measurements were recorded on ELICO CM-180 conductivity bridge in dry DMSO (10^{-6} M) solution using dip-type conductivity cell fitted with a platinum electrode. Thermal analysis (TG-DTA) of the metal complexes were recorded on Perkin Elmer STA 6000 thermal analyzer under the N_2 atmosphere from ambient temperature to 700 ^{o}C at a rate of heating 20 ^{o}C min^{-1}. Powder X-ray diffraction studies of the complexes were performed at

room temperature on Bruker AXS D8 Advance Diffractometer equipped with monochromatic Cu K_α radiation (λ= 1.5406 Å). Magnetic susceptibility measurements were made at room temperature on vibrating sample magnetometer (VSM) using Ni as calibrant.

*2.2 General method for the synthesis of ligand (**L**)*

A well stirred solution of 4-amino-N-(5-methyl-1, 2-oxazol-3-yl) benzene sulfonamide (**1**) (2.0 mmol) in 5-6 mL conc. HCl was cooled in an ice bath and diazotized with the solution of sodium nitrite (2.2 mmol) in 2 mL of conc. H_2SO_4. The resulting mixture was stirred for 2 h at 0-5 °C. The cold diazonium salt solution was added to the cold solution of 1-[1-phenyl-2-(phenylimino) ethyl] naphthalen-2-ol (**2**) (2.0 mmol) in acetic acid (10 mL). The reaction mixture was stirred for another 2 h at 0-5 ^oC (p^H 5-6). The crude product was filtered off, washed with hot water, dried and recrystallized from ethanol (**Scheme 1**).



Scheme 1: Synthesis of azo ligand (**L**)

2.3 Synthesis of Cu (II), Co (II) and Ni (II) complexes

To the hot methanolic solution of azo dye ligand **L** (0.3 mmol), a solution of appropriate metal acetates in methanol (0.16 mmol) was added dropwise with constant stirring. The reaction mixture was heated under reflux for 4 h and, the volume was reduced to half of the initial volume under reduced pressure. The resultant precipitate was filtered,

washed several times with distilled water and then dried in vacuum over anhydrous $CaCl_2$ desiccator (Yield: 60–70%).

2.4 Biological activity

The newly obtained ligand and its metal complexes were screened for their antibacterial activity against *Klebsiella pneumonia* (ATCC 13883), *Escherichia coli* (ATCC 25922) *and Bacillus subtilis* (ATCC 19659) by the well diffusion method. All bacterial strains were maintained on nutrient agar medium at ±37 °C. These cultures are obtained from the Department of Microbiology, Kuvempu University, Shimoga, Karnataka, India which are previously procured from Institute of Microbial Technology Chandigarh (IMTC), India. The stock solutions of the ligand and its metal complexes (1 mg/mL) were prepared by adding 10 mg of each compound in 10 mL freshly distilled DMSO. Further, the different concentrations of the test compounds (1.5 and 3.0 mg/mL), were prepared by diluting the stock solutions with the required amount of DMSO.

2.4.1 Antibacterial screening

The liquid medium containing the bacterial subcultures was autoclaved for 20 min at 15 lb pressure before inoculation. Mueller Hinton agar medium was used for the antibacterial studies. The pure dehydrated Mueller Hinton agar (38 g) was dissolved in 1000 mL of distilled water. The agar plates were prepared by using the above media and wells were dug with the help of 6 mm sterile metallic cork borer. Each plate was inoculated with 18 h old bacterial culture (100 μL) using a micropipette and spread uniformly using bent glass rod on each plate. The test compounds with different concentrations (1.5 and 3.0 mg/mL) in dry DMSO were added dropwise onto a 6 mm diameter filter paper disc placed in the centre of the agar plates. The plates were then kept for incubation at 36 °C for 24 h. After completion of the incubation period, the diameters of zone of inhibition were measured in millimeters by

using antibiotic zone scale procured from Hi-media. All the tests were performed in triplicate and, the average was taken as the final reading.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration where no visible turbidity was observed in the test tubes. The MIC of the test compounds was determined by Microtitre plate assay was performed as previously described by [**19, 20**] with slight modification. 100 µL of sterile growth media was pipetted into microtitre plate, and to it, 100 µL aliquot of synthesized compounds were then diluted two-fold in each well. Finally, 100 µL of the culture media of optical density (0.5) at 600 nm was added to all the wells. Positive control contained growth media inoculated with microorganisms. Negative control included synthesized compounds and sterile growth media only. The plates were incubated for 24 h at 37 ^{0}C and absorbance was recorded at 600 nm. The lowest concentration that produced no visible growth of the bacteria with the control was considered as MIC.

2.4.2 DPPH radical scavenging activity

Free radical-scavenging capacities of newly synthesized ligand and its complexes were determined according to the previously reported procedure [**21**]. The stock solution of the extracts (1 mg/mL) was prepared and DPPH (0.004%) using 95% of methanol. Freshly prepared DPPH solutions were taken in test tubes and, extracts are to be added (100 µg) to every test tube so that the final volume will be 3 mL and after 10 min, the absorbance will be read at 517 nm using the UV-Visible spectrophotometer. Ascorbic acid was used as the standard. The assay was carried out in triplicate and, the percentage of inhibition was calculated using the following formula,

$$\% \text{ Radical Scavenging activity} = \frac{[A_{br} - A_{ar}]}{A_{br}} \times 100 \quad (1)$$

Where A_{br} and A_{ar} are the absorbance's before and after the reaction has taken place.

2.4.3 DNA cleavage studies

The extent to which the newly synthesized azo dye ligand and its Cu (II), Co (II) and Ni (II) complexes could behave as DNA cleavage agents was studied by calf-thymus DNA (Cat. No 105850) as a target molecule. The efficiency of the target molecules as cleavage agents was examined by gel electrophoresis technique. The nutrient broth was used as the media (peptone 10, NaCl 10 and yeast extract 5 g L^{-1}). The gel electrophoresis experiment was done according to the following procedure [**22, 23**]. Briefly, 200 mg of agarose was dissolved in 25 mL of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/ 1 ltr) by boiling. When the gel attains approximately 55 ^{o}C, it was poured into the gel cassette fitted with a comb. The gel was allowed to solidify and then carefully the comb was removed. The gel was placed in the electrophoresis chamber flooded with TAE buffer. The target compounds were dissolved in freshly distilled DMSO (1 mg/mL). The test compounds were added separately to the isolated CT- DNA (225 ng) and incubated for 2 h at 37 ^{o}C. After incubation, 20 µL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the wells, along with standard DNA marker and constant electricity of 50 V was passed for around 45 min. The gel was removed and thoroughly strained with Ethidium bromide (ETBR) solution (10 µg/mL) for 10-15 min. The bands were observed under UV transilluminator (UVP, Germany) and photographed to determine the extent of DNA cleavage, and the results were compared with those of a standard DNA marker.

2.4.4 In silico molecular docking studies

To investigate the antimicrobial potential of the synthesized ligand and its metal complexes using *in silico* molecular docking method [**24**]. The structures of the target compounds were generated using Chem Bio Draw tool (Chem Bio Office Ultra 14.0 suite) assigned with proper two-dimensional orientation and structure of each was checked for structural drawing error and converted to 3D format followed by the minimization of energy using Ligprep in Schrodinger Maestro. The 3D coordinates of protein targets were obtained form 2XCT-Protein Data Bank (PDB) and subjected to energy minimization after assigning proper bond orders and ionization states along with charge fixing using Schrodinger maestro version 9.0. The best-docked conformation of the ligand and its metal complexes was identified based on glide energy, docking score and also by analysing the active site hydrogen bonds and hydrophobic interactions. PDBSUM was used for Ligplot representation of protein-ligand interactions and, ribbon/surface view representations of docked complexes were generated using PyMol.

3. Results and Discussion:

3.1 Chemistry

The orange-red colored azo dye ligand (**L**) is found to be soluble in chloroform and other common organic solvents like methanol, DMF, and DMSO. All the synthesized metal complexes are colored solids, non-hygroscopic, amorphous in nature, stable at room temperature and found to be soluble in DMF, and DMSO. Melting points of the newly synthesized complexes were found to be above 300 ^{o}C. The results of the elemental analysis of the ligand and its metal complexes were displayed in **Table 1**. Elemental analysis and analytical data of the complexes suggest that the metal to ligand ratio is 1:2 stoichiometry of the type [M $(L)_2$] for all the complexes, where **L** stands for the deprotonated ligand. The

molar conductance measurements suggest the non-electrolytic nature of the complexes in DMSO (10^{-6} M) [**25**].

<Insert Table 1>

3.2 IR spectral data

The main absorption bands of the ligand (**L**) and its Cu (II), Co (II) and Ni (II) complexes are displayed in **Table 2**. On complexation, the band at 1492 cm^{-1} for the azo group in the free ligand was shifted to a lower wavelength in the range ~1589–1573 cm^{-1}, indicating the coordination of the nitrogen atom of the azo group to the metal ion [**26**]. The broad absorption band at 3278 cm^{-1} for phenolic OH group of the free ligand has been disappeared in all the metal chelates indicates the formation of bonds between the metal ion and phenolic oxygen atom *via* deprotonation. The phenolic C-O functional group of the ligand exhibited absorption band at 1237 cm^{-1} which are shifted to higher frequencies, i.e., 1259-1266 cm^{-1} in all the metal complexes [**27**]. This confirms the formation of M-C-O bond in all the complexes. The absorption band due to NH and azomethine (-CH=N-) function in metal complexes of ligand have displayed a bands in the region 3166-3134 cm^{-1} and 1620-1578 cm^{-1} respectively, which have appeared at about the same region as in the case of ligand thus confirming the non-involvement of NH and azomethine function in coordination with the metal ions. But in the case of Co (II) complex, the absorption due to azomethine functional group was shifted to a lower wavelength with a difference of ~42 cm^{-1} indicating the involvement of nitrogen atom of the azomethine group. Further, the spectrum of all the complexes shows new bands in the region 462-480 cm^{-1} and 587-590 cm^{-1} due to the formation of M-N and M-O bonds respectively [**28, 29**].

<Insert Table 2>

3.3 Electronic absorption spectral data

Electronic spectra of the synthesized azo dye ligand and its metal complexes were recorded in DMSO solution, and the absorption bands are displayed in **Table 3**. The spectrum of the ligand showed an intense band at 20, 618 cm^{-1} corresponds to the $n\rightarrow\pi^*$ transition of azo group. In the electronic spectrum of the Cu (II) complex exhibited bands at 29, 205 and 20, 104 cm^{-1} which are assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transitions characteristics of square planar geometry [**30, 31**]. The Co (II) complex displayed three absorption bands at 19607, 26595 and 31152 cm^{-1} characteristics of tetrahedral geometry, which may be assigned for $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), $^4T_{1g} \rightarrow {}^4A_{2g}$ (F) and $^4T_{1g} \rightarrow {}^4T_{1g}$ (P) transitions respectively [**32**]. From the electronic spectrum of the Ni (II) complex, it is observed that the bands at 19762, 26109 and 31847 cm^{-1} are assigned to $^1A_{1g} \rightarrow {}^1B_{1g,}$ $^1A_{1g} \rightarrow {}^1A_{2g}$, and INCT respectively. All these observations suggest the square planar geometry for Ni (II) complex [**33**].

<Insert Table 3>

3.4 ^1H NMR spectral data

The proton NMR spectra is an excellent tool to check the purity of the organic compounds. The ^1H NMR spectrum of the ligand (**L**) was measured in DMSO- d_6 at room temperature. The azo dye ligand displayed a singlet at 15.62 ppm (s, 1H, Ar-OH) is due to hydroxyl group attached to naphthol ring. The signal due to NH proton attached to the sulfamethoxazole moiety resonated as a singlet at 11.29 ppm (s, 1H, NH). The signal appeared at 8.76 ppm as a singlet assigned for azomethine proton (s, 1H, CH=N). The signals due to twenty aromatic protons (m, 20H, Ar-H) have resonated as multiplets in the region 8.43-6.70 ppm. A proton resonated at 6.13 ppm as singlet may be assigned to aliphatic CH at the junction (s, 1H, aliphatic CH) [**34**]. Another singlet observed at 2.32 ppm (s, 3H, CH_3) corresponds to methyl protons attached to the thiazole ring.

3.5 Mass spectral data

The ESI mass spectra of the azo dye ligand and its Cu (II), Co (II) and Ni (II) complexes are recorded and investigated at 70 eV electron energy. The spectral data showed that the molecular ion peaks are in good agreement with their suggested empirical formula. The mass spectrum of the azo dye ligand **L (**supplementary data, Fig. S1**)** showed molecular ion peak due to M^+ at m/z 603 which corresponds to the molecular weight of the azo dye ligand. The mass spectra of Cu (II), Co (II) and Ni (II) complexes of ligand **L** exhibited molecular ion peaks due to M^+ at 1266, 1261 and 1260 which are equivalent of their molecular mass of the respective complexes. The tentative mass spectral fragmentation pattern of the above metal complexes is presented in the **Schemes 2-4** and are provided in the supplementary data.

3.6 Magnetic susceptibility measurements

The magnetic susceptibility is an important technique which mainly focuses on the electronic structure of the metal complexes formed by the coordination of the ligand with the different metal ions [**35**]. The magnetic moments of the newly synthesized complexes were measured at room temperature for the Cu (II), Co (II) and Ni (II) complexes are listed in **Table 3**. The Cu (II) complex showed magnetic moment corresponding to one unpaired electron which is 1.86 BM with a slight orbital contribution to the spin only value 1.73 BM and the absence of spin-spin interaction in the complex accounting for the possibility of square planar geometry [**36**]. The magnetic moment for Co (II) complex is 4.98 BM corresponding to three unpaired electrons which is well within the expected range 4.70-5.20 BM, suggesting the consistency with its octahedral geometry [**37**]. The Ni (II) complex was observed to be diamagnetic which corresponds to square planar geometry around Ni (II) ion [**38, 39**].

3.7 Thermal data of the complexes

The thermal behaviour of Cu (II), Co (II) and Ni (II) complexes was carried out to confirm their molecular structures and thermal stability. Thermal degradation of the complexes was studied at a rating of heating 20 ^{o}C min^{-1} under nitrogen atmosphere within temperature range 20-750 ^{o}C. The tentative thermal decomposition of the metal complexes with respect to temperature and formation of the respective metal oxides are displayed in **Table T-1** and provided in supplementary material.

The Cu (II) complex (**Fig. 1**) of the azo dye ligand (**L**) showed degradation four stages. The first step occurred in the temperature range 316-344 ^{o}C, with a weight loss of 11.49% (calcd. 12.97%) due to the removal of two moles of C_4H_4NO fragment. The second degradation stage of estimated mass loss 13.06% (calcd. 12.49%) within the temperature range 344-421 ^{o}C due to the elimination of two moles of SO_2NH fragment in the complex. The third stage of degradation occurred within the temperature range 421-483 ^{o}C with a weight loss of 30.30% (calcd. 30.71%) due to the removal of the two moles of $C_{14}H_{13}N$ fragment. The final stage of degradation occurred with a mass loss of 33.67% (calcd. 33.71%) due to the elimination of $C_{14}H_{11}N_2O$ fragment within the temperature range 483-721 ^{o}C. The weight of the residue corresponds to the one mole of cupric oxide and four moles of carbon.

In the thermogram of the Co (II) complex, the first step of decomposition represents the mass loss due to two moles of phenyl groups at 253-368 ^{o}C with a weight loss of 12.33% (calcd. 12.21%). The second decomposition occurred at 368-631 ^{o}C with a practical weight loss of 59.37% (calcd. 60.30%), due to the elimination of two moles of a $C_{18}H_{14}N_5SO_3$ fragment. In the final step of decomposition, the complex showed gradual decomposition up

to 726 ^{o}C due to the removal of remaining organic moiety with a practical weight loss of 27.98% (calcd. 21.10%). The weight of the residue corresponds to one mole of CoO. Similarly, the Ni (II) complex undergo thermal decomposition in four steps in temperature ranges at 256-329 ^{o}C, 329-441 ^{o}C, 441-507 ^{o}C, and 507-726 ^{o}C respectively. The weight loss in 1st 2.88% (calcd. 2.38%), 2nd 31.06% (calcd. 30.83%), 3rd 12.46% (calcd. 13.18%) and 4th 36.99% (calcd. 37.81%) were tentatively assigned to the elimination of $2CH_3$, $2C_{14}H_{12}N$, $2C_3H_3N_2O$ and $C_{16}H_{10}N_2O$ fragments respectively leaving NiO as residue.

< Insert Figure 1>

3.8 Powder X-ray diffraction studies

The synthesized metal complexes were soluble in some polar solvents, but crystals that are suitable for single-crystal studies were not obtained. Therefore, Powder X-ray diffraction patterns of Cu (II), Co (II) and Ni (II) complexes were obtained to study the degree of crystallinity of the complexes. Powder X-ray diffraction pattern for Cu (II) complex (**Fig. 2**) showed 5 reflections in the range of 9-25^{o} (2θ). These reflections are obtained due to the diffraction of X-ray by the planes of complexes. The inter-planer spacing (d) has been calculated from the positions of intense peaks using Bragg's equation $n\lambda = 2d \sin\theta$ (where λ= 1.5406 Å). The obtained and calculated inter-planer spacing (d) are consistent and are displayed in **Table 4.** Further, the unit cell calculations were calculated for cubic symmetry by all the obtained peaks, and $h^2 + k^2 + l^2$ values were determined. The obtained and calculated inter-planer d-spacing values were found to be in good agreement. The $h^2 + k^2 + l^2$ values of Cu (II) complexes are 1, 3, 4, 6 and 7. The lattice parameter for Cu (II) complex is calculated and found to be $a = b = c$ = 9.43. From the results, it is evident that the presence of forbidden number 7 indicates that the Cu (II) complex may belong to hexagonal or tetragonal systems.

Similar calculations were carried out for Co (II) and Ni (II) complexes and displayed 7 and 25 reflections in the range 6-43^{o} (2θ) respectively. The major peaks have been identified and observed inter-planer spacing (d) had been compared with the calculated ones. The unit cell calculations were obtained, and the $h^2 + k^2 + l^2$ values were determined. The $h^2 + k^2 + l^2$ values for Co (II) complexes are 1, 2, 3, 5, 6 and for Ni (II) complex are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 18, 22, 25, 27, 30, 34, 40. The presence of forbidden numbers like 7 for Co (II) complex and 15 for Ni (II) complex indicates both the complexes may belong to hexagonal or tetragonal systems. The calculated lattice parameter for Co (II) and Ni (II) complexes are $a = b = c$ = 8.66 and 13.16 respectively.

<Insert Table 4>

< Insert Figure 2>

3.9 Pharmacological studies

3.9.1 Antimicrobial activity

The *in-vitro* antibacterial studies of novel azo dye ligand and its metal complexes were screened against bacterial strains *K. pneumonia, E. coli and B. subtilis* and results are presented in **Table 5**. From the results, it is noticed that all the metal complexes exhibited better activity compared to the free ligand. The enhancement of activity on the coordination of metal ion with the free ligand is attributed to the increase in the lipophilic character of the complexes arising from chelation, which mainly decreases the polarity of the metal ion. This is mainly because of π-electron delocalization within the chelating ring and partial sharing of positive charge of the metal ions with the donor groups of the ligand [**40**]. Increase in the lipophilic nature of the metal ion on chelation favours the permeation through the lipid layer of the cell wall. Moreover, the mode of action of the target compounds involves the formation of hydrogen bonds with the nitrogen atom of the azo group with the active enzyme

sites of the cell, resulting in the interference with the normal cell processes. These complexes also disturb the respiration process of the cell and thus block the protein synthesis, which restricts further growth of the organism. Some other factors such as solubility, conductivity, bond length, dipole moment and permeability also increase the activity. [**41**]. Further, it is observed that the Co (II) complex showed higher MIC value than Cu (II) and Ni (II) complexes.

<Insert Table 5>

3.9.2 DPPH radical scavenging activity

Antioxidants are the compounds that fight and prevent specific metabolic disorders caused by free radical-induced oxidative stress. The synthetic antioxidants have been found to have more antioxidant activities compared to naturally occurring antioxidants. However, the use of synthetic antioxidants is limited due to their toxicity. In this regard, there is a significant interest to find a new class of synthetic compounds which are less toxic and do not show any pathological side effects [**42, 43**]. Therefore, in the present study, the azo dye ligand and its metal complexes were screened for their scavenging activity using DPPH assay. The scavenging effect of the tested compounds at different concentrations is demonstrated in **Fig. 3**. And it is evident that the activity is concertation dependent. Among the tested compounds, Cu (II) and Co (II) complex showed good scavenging activity, whereas azo dye ligand and its Ni (II) complex exhibited moderate activity compared with standard ascorbic acid.

< Insert Figure 3>

3.9.3 DNA cleavage studies

. The DNA cleavage is an efficient tool used to design effective drugs that are mainly targeted to DNA [**44**]. Therefore, in the present study, we examined the interaction between the metal complexes of the azo dye ligand (**L**) with calf-thymus DNA using gel electrophoresis technique. And it is based on the migration of DNA molecule under the impact of electric potential. The gel picture showing the cleavage of CT-DNA is displayed in **Fig. 4**. From the analysis of electrophoretic results, it is noted that there was a difference in the migration of the lanes of ligand and its metal complexes as compared to the control CT-DNA at 100 µg L^{-1} concentration. Therefore, it is evident that the ligand and its metal complexes exhibited complete cleavage of tested DNA. Thus, this study shows that the control DNA alone does not show any apparent cleavage, whereas the ligand and its complexes do show. As the synthesized ligand and its metal complexes were observed to cleave the DNA, it can be concluded that the tested compounds competently inhibit the growth of the microorganisms by cleaving CT DNA [**45**].

< Insert Figure 4>

3.9.4 In silico docking results

Molecular docking is a key tool in the computer drug design and directs to achieve relative orientation between the target protein and the drug such that the free energy of the overall system is minimized. In order understand the mechanism of drug action, the molecular docking studies were carried out for the synthesized compounds with the target receptor glucose-amine-6-phosphate synthase [**46, 47**]. The docking results exhibited a possible interaction between the tested compounds and the receptor. The azo dye ligand and its metal complexes displayed well-established bonds with amino acids (Gly436, Asp437, Ser438, Arg458, Gly459, Asp510, His1081, Arg1122, Phe1123, Lys460, Ile461, Glu477, Ser1084, Glu1088, Asp512, and Gly1082) in the target enzyme active pockets (**Fig. 5**). All

the compounds showed noticeable results with favourable binding energy and showed the bonding with one or the other amino acids in the active sites. From the results it is evident that all the synthesized molecules exhibited potential binding energy with a strong affinity towards the target enzyme glucose-amine-6-phosphate synthase ranging from -5.6 to -7.1 kJ/mol and presented in **Table 6**.

<Insert Table 6>

< Insert Figure 5>

Conclusions

In the present study, three new Cu (II), Co (II) and Ni (II) complexes of the azo dye ligand (**L**) derived from sulfamethoxazole were synthesized and characterized by different analytical and spectroscopic techniques. The analytical data revealed that the metal to ligand stoichiometry in all the synthesized complexes is 1:2. Spectral analysis and magnetic susceptibility measurements suggested square planar geometry for Cu (II) and Ni (II) complexes and octahedral environment for Co (II) complex. Based on these physicochemical evidences, following structures were proposed for the metal complexes (**Fig. 6**). The azo dye ligand (**L**) and its metal complexes are proved to have significant antimicrobial activity against tested pathogens. Among the tested compounds, Co (II) complex exhibited higher antibacterial activity than Cu (II) and Ni (II) complexes. The antioxidant potential of the compounds was examined by DPPH radical scavenging assay. All the metal complexes showed promising antioxidant activity than the free ligand. DNA cleavage activity of the synthesized compounds was studied against CT-DNA, and the tested compounds are completely cleaved the DNA. The molecular docking studies were carried out to explore the binding modes and the possible interaction with the target enzyme glucose-amine-6-phosphate synthase. Docking studies revealed that the tested compounds fit into the receptor active site with favourable binding energy. Moreover, all

the tested compounds were able to form H-bonds with the various amino acids in the enzyme active sites.

< Insert Figure 6>

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Table 1: Physical, analytical and molar conductance data of ligand (**L**) and its metal complexes.

Ligand/Complexes		Mol. Formula	Mol. Wt.	M.P. ^{o}C	Color	Elemental analysis (%) Calcd. (found)				λ_m $cm^2\ \Omega^{-1}\ mol^{-1}$
						C	H	N	M	
L	L	$C_{34}H_{27}N_5O_4S$	601.67	269	Light orange	67.87 (67.22)	4.52 (4.35)	11.64 (11.13)	-	-
L1	$[Cu\ (L)_2]$	$C_{68}H_{52}CuN_{10}O_8S_2$	1264.87	>300	Dark blue	64.57 (64.12)	4.14 (4.18)	11.07 (10.95)	5.02 (5.12)	22
L2	$[Co(L)_2]$	$C_{68}H_{52}CoN_{10}O_8S_2$	1260.26	>300	Dark red	64.81 (64.62)	4.16 (3.99)	11.11 (11.18)	4.68 (4.21)	19
L3	$[Ni(L)_2]$	$C_{68}H_{52}NiN_{10}O_8S_2$	1260.02	>300	Brown	64.82 (64.44)	4.16 (4.12)	11.12 (11.20)	4.66 (4.18)	18

Table 2: IR spectral data of azo dye ligand (**L**) and its metal complexes (cm^{-1})

Ligand/Complexes		υ_{OH} (phenolic)	υ_{NH}	$\upsilon_{N=N}$ (azo)	$\upsilon_{CH=N}$ (azo methine)	υ_{C-O} (phenolic)	υ_{M-N}	υ_{M-O}
L	L	3278	3143	1492	1620	1237	-	-
L1	$[Cu\ (L)_2]$	-	3134	1471	1614	1262	480	587
L2	$[Co(L)_2]$	-	3166	1463	1578	1259	476	586
L3	$[Ni(L)_2]$	-	3161	1477	1612	1266	462	590

Table 3: Electronic spectral data of ligand (**L**) and its complexes.

Ligand/Complexes		Absorption in cm^{-1}	Transition	μ_{eff} (BM)	Geometry
L	*L*	20,618	n→ π*	-	-
L1	*[Cu (L)$_2$]*	20,104	$^2B_{1g} \rightarrow {}^2A_{1g}$	1.86	Square-planar
		29,205	INCT		
L2	[Co(L)$_2$]	19,607	$^4T_{1g} \rightarrow {}^4T_{2g}$ (F)	4.98	Octahedral
		26,595	$^4T_{1g} \rightarrow {}^4A_{2g}$ (F)		
		31,152	$^4T_{1g} \rightarrow {}^4T_{1g}$ (P)		
L3	[Ni(L)$_2$]	19,762	$^1A_{1g} \rightarrow {}^1B_{1g}$	Diamagnetic	Square-planar
		26,109	$^1A_{1g} \rightarrow {}^1A_{2g}$		
		31,847	INCT		

Table 4: Powder X-ray diffraction data of Cu (II) complex of the azo dye ligand (**L**)

Peak no.	2θ	θ	Sin θ	Sin^2 θ	1000 Sin^2 θ	$h^2 + k^2 + l^2$ (Sin^2 θ/CF)	(h k l)	d		a in Å
								Obs.	Calcd.	
1.	9.375	4.687	0.0817	0.0066	6.67	1 (1)	100	9.433	9.428	9.426
2.	15.831	7.915	0.1377	0.0189	18.96	2.84 (3)	111	5.598	5.594	9.441
3.	18.682	9.341	0.1623	0.0263	26.34	3.94 (4)	200	4.749	4.746	9.428
4.	22.903	11.451	0.1985	0.0394	39.40	5.90 (6)	211	3.882	3.880	9.426
5.	25.048	12.524	0.2168	0.0470	47.00	7.04 (7)	-	3.555	3.553	9.427

Table 5: Minimum inhibitory concentration (MIC, mg/mL) of the azo dye ligand (**L**) and its metal complexes

Organisms	**L**	**L1**	**L2**	**L3**	**Std (Tetracycline)**
Klebsiella pneumoniae	36	38	41	38	39.06
Escherichia coli	7.5	8.62	9.02	9.85	9.53
Bacillus subtilis	7.2	10.01	9.62	10.13	9.53

Table 6: Results obtained in molecular docking study for azo dye ligand (**L**) and its metal complexes with receptor glucose-amine-6-phosphate synthase.

Compounds	Affinity (kcal/mol)	H-bonds	H-bond length (å)	H-bond with	Hydrophobic interactions
L	-7.1	2	3.14 3.24	2XCT:Gly1082::L:O3 2XCT:Glu435::L:N2	Gly436, Asp437, Ser438, Arg458, Gly459, Asp510, His1081, Arg1122, Phe1123
L1	-5.6	3	2.80 3.06 3.14	2XCT:Gly459::L1:O6 2XCT:Asp437::L1:O7 2XCT:Asn476::L1:O5	Gly436, Arg458, Lys460, Ile461, Glu477, Ser1084, Arg1122
L2	-6.0	2	2.80 2.98	2XCT:Asp512::L2:N8 2XCT:His1081::L2:O5	Arg458, Gly459, Lys460, Ser1084, Glu1088, Arg1122, Phe1123
L3	-6.9	-	-	-	Gly436, Asp437, Arg458, Gly459, Asp512, Lys460, Glu477, Gly1082, Ser1084, Arg1122, Phe1123, Ser1084, Glu1088

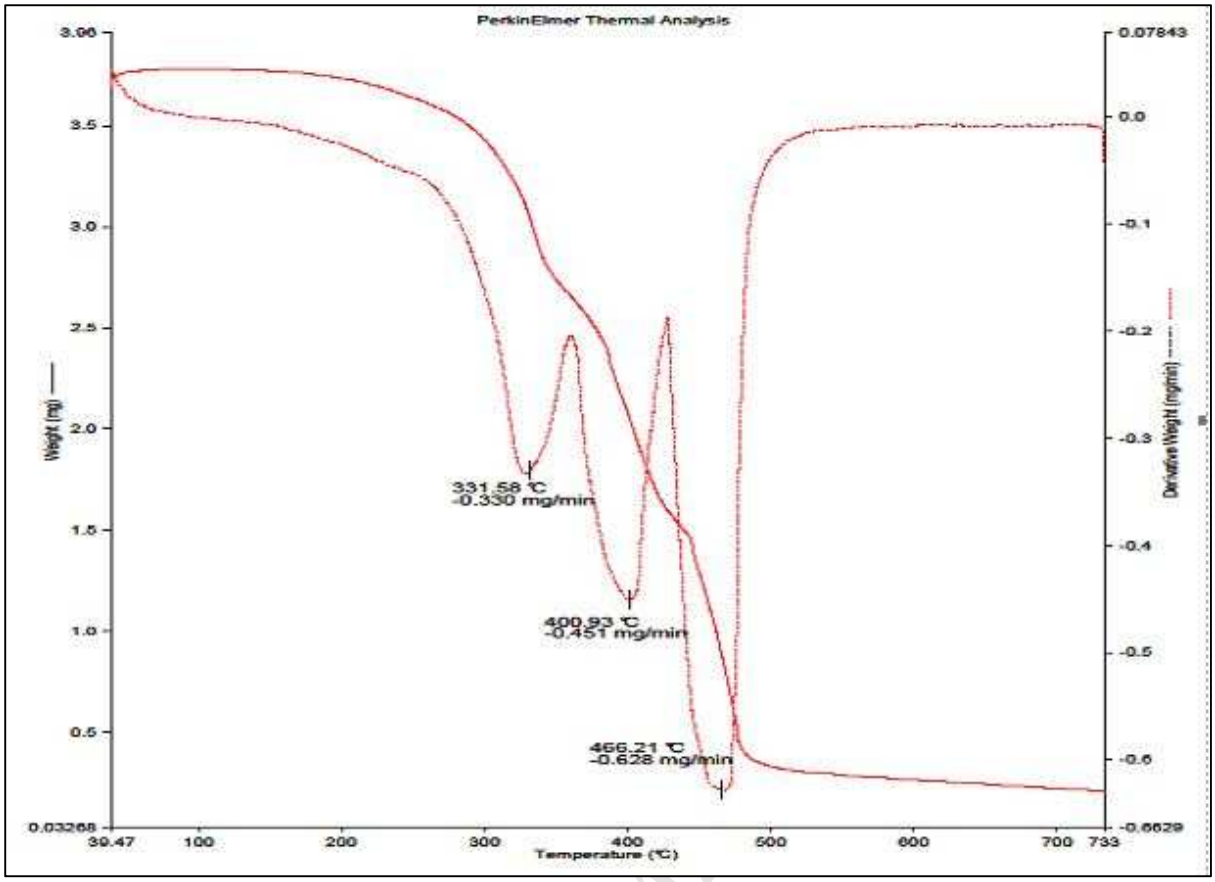


Fig. 1: TG-DTA curve for Cu (II) complex of the azo dye ligand (**L**)

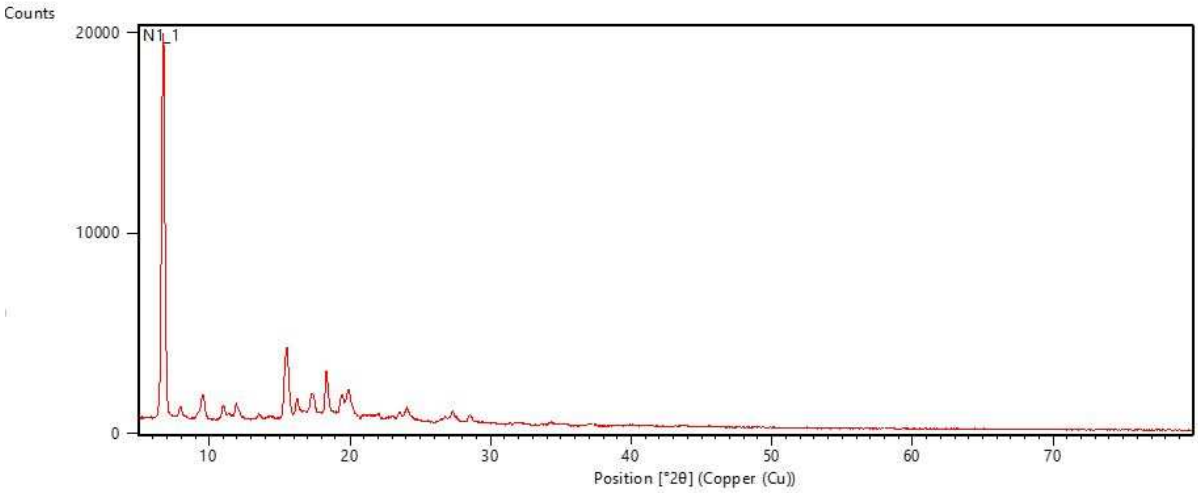


Fig. 2: Powder X-ray spectra of Cu (II) complex

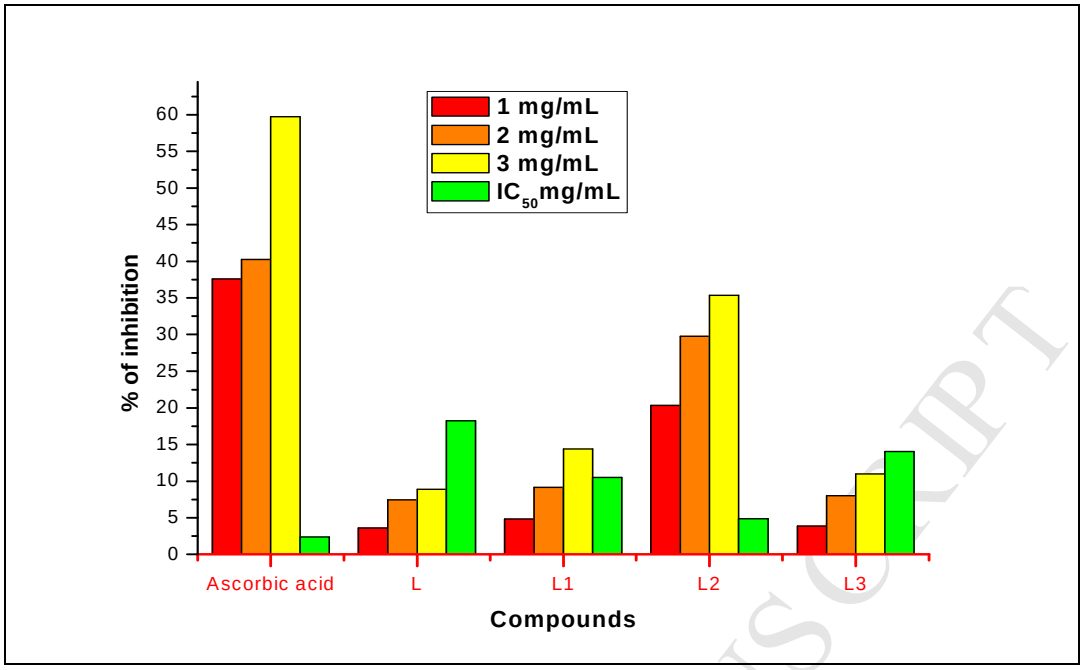


Fig. 3: DPPH radical scavenging activity of azo dye ligand (**L**) and its metal complexes

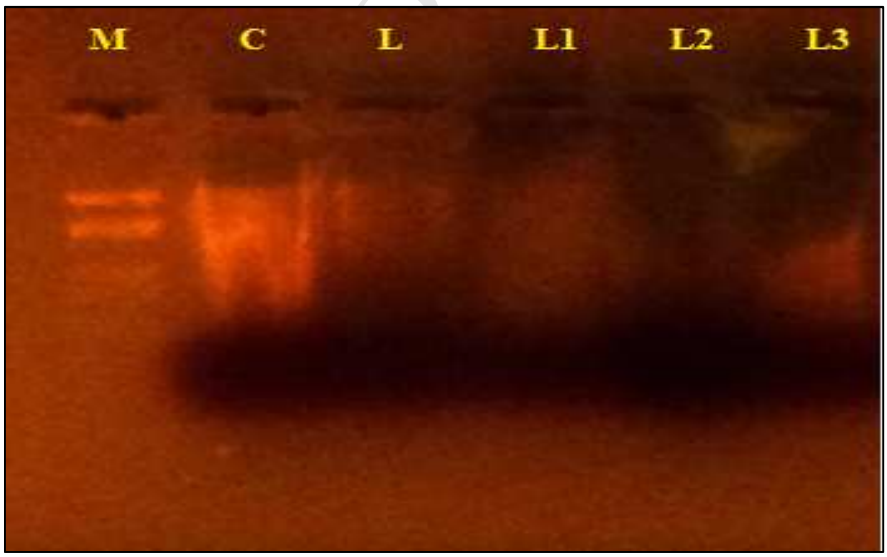


Fig. 4: DNA cleavage activity on Calf-thymus DNA: **M**: Standard DNA, **C**: Control DNA (untreated Calf-thymus DNA), **L**: azo dye ligand, **L1**: Cu (II) complex, **L2**: Co (II) complex and **L3**: Ni (II) complex.

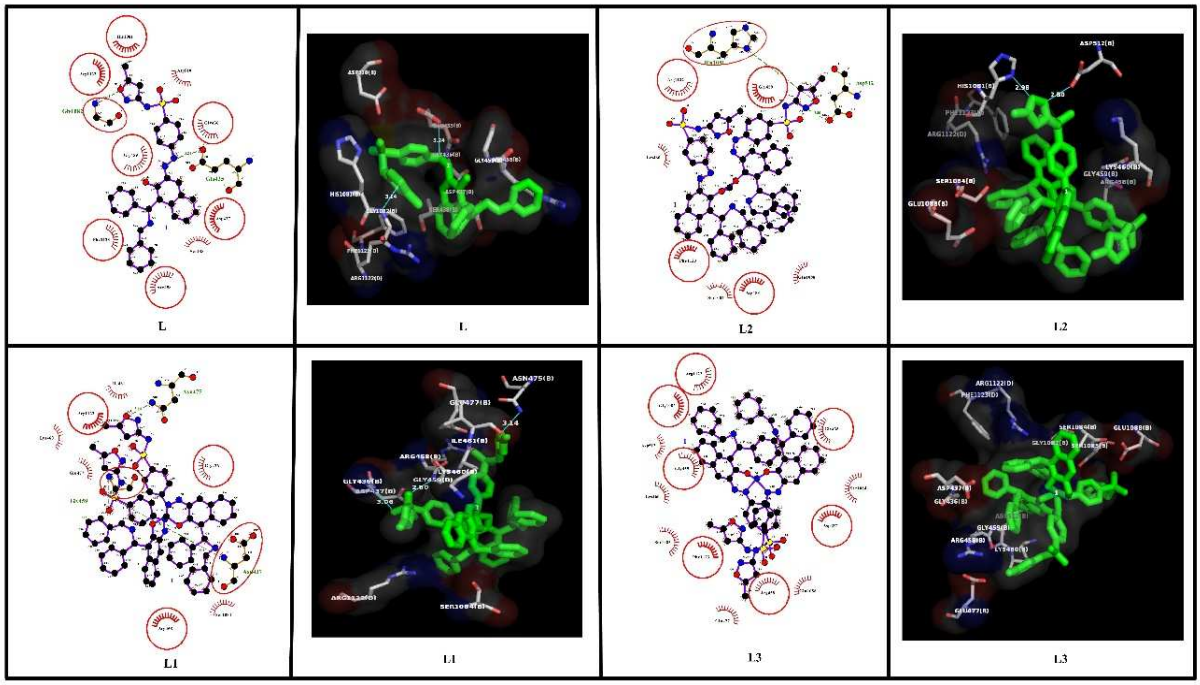
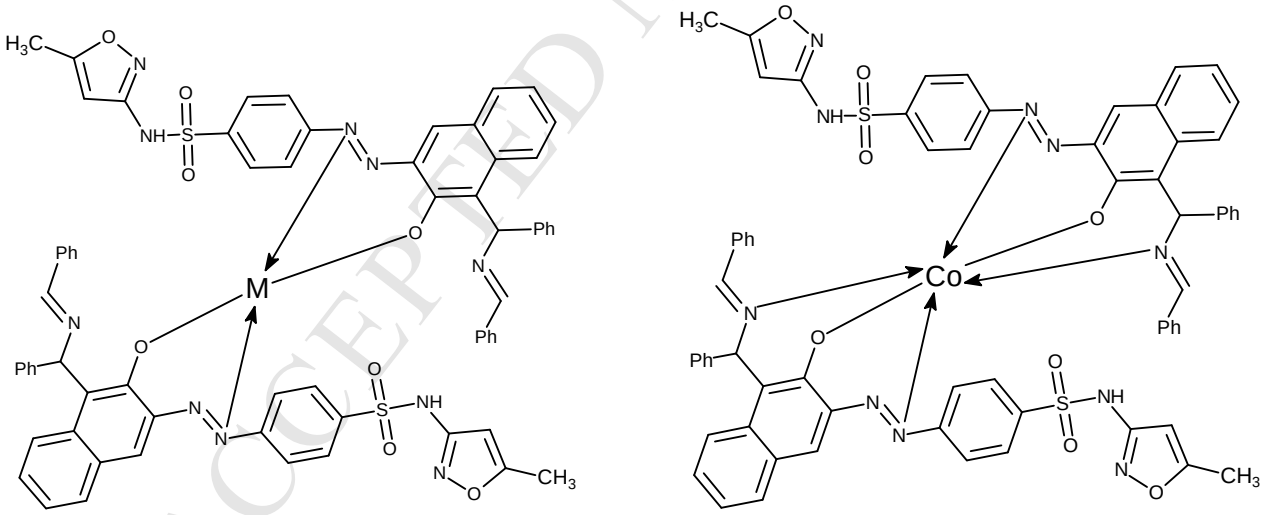


Fig. 5: *In silico* molecular docking studies of azo dye ligand (**L**) and its metal complexes



Where M = Cu (II) and Ni (II)

Fig. 6: Proposed structure of the Cu (II), Co (II) and Ni (II) complexes of the azo dye ligand (**L**)

Heighlights of the research work

- Synthesis and spectroscopic characterization of novel bioactive Cu (II), Co (II) and Ni (II) complexes derived from sulfamethoxazole based azo dye.
- The elemental analysis and analytical data confirmed the metal to ligand ratio is 1:2 stoichiometry of the type [M $(L)_2$].
- All the synthesized compounds showed significant antimicrobial activity.
- The ligand and its metal chelates have promising cleavage ability against Calf Thymus DNA.