Synthesis, characterization and biological evaluation studies of Cu(II) and Zn(II) complexes with gly-o-andn or gly-p-andn as primary ligand and N, N' donors as secondary ligand

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Abstract

Metal complexes with heterocyclic Schiff bases are of substantial curiosity for the chemists. Ligand modifications are easily accessible due to preparative simplicity, structural variability and tunable electronic properties. Hetero atoms like N and O when incorporated in Schiff bases play a key role at binding sites in metallobiomolecules. Transition metals form an integral part of biological system and their mixed ligand complexes with Schiff bases have established their remarkable application in biocidal fields as antibacterial, antifungal, anti-inflammatory and as anticancerous agents. Keeping in mind their marked biological activity, this work reports the synthesis and characterization of
mixed ligand complexes of zinc and copper with Schiff base (obtained by the condensation of glyoxal with ortho and para anisidine) as primary and N, N' donor molecules as secondary ligands.

1. Introduction

Since their identification in nineteenth century, coordination complexes had been a major challenge for the inorganic chemists as they do not follow the standard rules of valence. This problem has been resolved by Alfred Werner (a Swiss chemist) in the year 1913, as he has given the most successful theory explaining the structure of these complexes [1,2]. Today they embrace a large quantity of inorganic researches. Variation in the coordination number, design of ligand, steric environment and chelation around central metal ion not only alters the thermodynamic and kinetic properties but also brings drastic amendments among biological properties of both metal and ligand moieties[3,4]. Since the late 3d transition metals like Co(II), Ni(II), Cu(II) and Zn(II) are electron rich species and become easily soluble in biological fluids, so the interest of the chemists have been shifted from platinum based drugs to non platinum based therapeutics. Copper is one of the essential trace elements present in most of the tissues of the body. A large number of copper dependent enzymes are found in biological systems e.g. Cytochrome C oxidase is required for cellular utilization of oxygen, Angigenin for stimulation of blood vessels construction etc. [5]. Zinc, next to iron, is present as an essential structural component in enzymes and can also be located at their active sites [6]. It acts as a Lewis acid and owing to its small radius to charge ratio it forms quite strong coordinate covalent bonds with ligands containing nitrogen and oxygen as donor atom [7,8].

Imines or Schiff bases were named after Italian naturalized chemist Hugo Schiff who first synthesized them in 1864 [9]. They are nitrogen analogs of aldehyde or ketone containing –
C=N- group where nitrogen is attached to aryl or alkyl group and not to hydrogen atom. Owing to their greater flexibility, sensitivity and selectivity they found large number of widely accepted applications in the area of biological, analytical, nuclear, pharmacological, radio immunotherapy, clinical and biochemical fields [10 - 20]. Hetero atoms like N and O when incorporated in Schiff bases play a key role at binding sites in metallobiomolecules. Diimines i.e. 2,2'-bipyridine and 1,10-phenanthroline are biochemically active, π - electron deficient and nitrogen containing chiral bidentate ligands [21]. These ligands can stabilize low oxidation state transition metal ions and endow complexes with hydrophobic properties with inbuilt M-N bond strength complemented by the chelate effect [22, 23]. The specific pharmacological activity of transition metal complexes with these N, N' donor ligands can be characterized by their enhanced antibacterial, antiviral, antitumor, antifungal activities [6, 24 - 27].

In order to frame enhanced metal based therapeutics, it is essential to determine and analyze the drug – protein relations. These drugs are transported into the blood through a protein known as albumin. By reversible mode of binding it transports and delivers large number of exogenous and endogenous compounds like bilirubin, hormones, fatty acids, porphyrins, amino acids, drugs etc. [28 - 30]. Researches on the study of interaction of these serum proteins with drugs can help to attain pharmodynamic and pharmokinetic information [31 - 33]. So the present study concerns with the aim to synthesize metal complexes of zinc and copper with heterocyclic Schiff base (obtained by the condensation of glyoxal with ortho and para anisidine) as primary and N, N' donor molecules as secondary ligands. They were then analyzed for their antimicrobial activities against two bacterial and two fungal strains strains. The complexes were also analyzed for their interaction with BSA by UV titration method.
The studies would be helpful in exploring the potential of synthesized complexes as antimicrobial agents and to determine their mode of binding with serum proteins.

2. Experimental

2.1. Materials

Loba chemie provided the following chemicals; \( o \)- anisidine, \( p \)- anisidine 1,10-phenanthroline, 2,2-bipyridine, tris buffer and, zinc chloride. CDH provided glyoxal and from SDFCL, BSA was obtained. Double distilled water was used to prepare the tris buffer solution.

2.2. Physical Measurements

Shimadzu UV-1800(200-800) was used for recording of the UV-visible absorption spectra and on a Nicolet SHIMADZU FTIR 8400S spectrometer, IR spectra were recorded in the range of 4000-400 cm\(^{-1}\). BRUCKER ADVANCE I.I 400 NMR Spectrometer has been used for recording \(^1\)H NMR spectra using d\(^6\) – DMSO / CDCl\(_3\) as solvents with TMS as the internal reference. XEVO G2-XS QTOF Mass Spectrometer has been used for recording mass spectra using DMSO / Chloroform as solvent. Open capillary tubes are used to measure the melting points and were uncorrected. The well diffusion method was used for measuring antimicrobial properties.

2.3. Preparation of the Metal Complexes

The overall scheme for the synthesis of Schiff base of glyoxal and ortho / para - anisidine and their metal complexes with Cu(II) or Zn(II) and diimines is as follows:
2.3.1 Methodology for the synthesis of (gly-o-andn) Schiff base (L₁)

In hot methanolic solution (35 ml) of glyoxal (4 mmol, 0.232 g) was added drop wise a hot methanolic solution (35 ml) of o-anisidine (8 mmol, 0.985 g or 0.9 ml). The whole set up was placed into an oil bath for 5 h at 70°C. The clear solution thus obtained was allowed to evaporate slowly. After 82 h, brown colored crystalline product separates out; this was filtered and dried in desiccators. Yield: 79 %, Color: Brown, M.P. 72°C, UV ($\lambda_{\text{max}}$): 239 nm, 284 nm, MS: [M]$^+$ 268, Main IR peaks (cm$^{-1}$): $\nu$(C₆H₅ stretch) 3045, $\nu$(C=N) 1599, $\nu$(C–C) 1506, $^1$H NMR (400 MHz, CDCl₃), $\delta$ = 8.15 (s, 2H, -CH=N), 7.66 (d, 4H, Ar -H), 7.53 (d, 4H, Ar-H), 3.85 (s, 6H, -OCH₃).

Fig. 1: Scheme for the synthesis of Schiff base ligand and its metal complexes with Cu(II) and Zn(II) metal ions

Fig. 2: Scheme for synthesis of Schiff base (L₁)
2.3.2 Methodology for the synthesis of [Zn(L1)phen]Cl2 (1)

In hot methanolic solution (30 ml) of Schiff base (1 mmol, 0.268 g) (L1) was added methanolic solution (30 ml) of ZnCl2 (1 mmol, 0.136 g) with constant stirring at 70°C. The solution was refluxed for 10 h. A hot methanolic solution of 1,10-phenanthroline (1 mmol or 0.198 g) was added drop wise to above solution with refluxing continuing for further 8 h. White precipitates were collected after filtration. Several washings were made with cold methanol. Yield: 54 %, Color: White, M.P. Above 280°C, UV (λmax): 221 nm, 265 nm, MS: [M]+ 620, Main IR peaks (cm⁻¹): ν(OH) 3200 - 3400, ν(CH₅ stretch) 3047, υ(C=N) 1581, υ(C–C) 1516, υ(M-N) 426.

2.3.3 Methodology for the synthesis of [Zn(L1)bpy]Cl2 (2)

In hot methanolic solution (30 ml) of Schiff base (1 mmol, 0.268 g) (L1) was added methanolic solution (30 ml) of ZnCl2 (1 mmol, 0.136 g) with constant stirring at 70°C. The solution was refluxed for 10 h. A hot methanolic solution of 2,2'-bipyridine (1 mmol or 0.156 g) was added drop wise to above solution with further refluxing for 8 h. White precipitates were collected after filtration. Several washings were made with cold methanol. Yield: 52 %, Color: White, M.P. Above 280°C, UV (λmax): 239 nm, 291 nm, MS: [M]+ 578, Main IR peaks (cm⁻¹): ν(OH) 3200-3400, ν(CH₅ stretch) 3061, υ(C=N) + υ(C–C) 1595, υ(M-N) 412.

2.3.4 Methodology for the synthesis of [Cu(L1)phen]Cl2 (3)

In hot methanolic solution (30 ml) of Schiff base (1 mmol, 0.268 g) (L1) was added methanolic solution (30 ml) of CuCl2.2H₂O (1 mmol, 0.170 g) with constant stirring at 70°C. The solution was refluxed for 10 h. A hot methanolic solution of 1,10-phenanthroline (1 mmol or 0.198 g) was added drop wise to above solution with refluxing continuing for further 8 h. Green precipitates were collected after filtration. Several washings were made with cold methanol. Yield: 49 %, Color: Green, M.P. Starts decomposing at 262°C, UV (λmax): 269 nm, MS: [M]+ 602, Main IR peaks (cm⁻¹): ν(OH) 3200-3400, ν(CH₅ stretch) 3048, υ(C=N) 1581, υ(C–C) 1512, υ(M-N) 428.
2.3.5 Methodology for the synthesis of \([\text{Cu}(L_1)\text{bpy}]\text{Cl}_2\) (4)

In hot methanolic solution (30 ml) of Schiff base (1 mmol, 0.268 g) \((L_1)\) was added methanolic solution (30 ml) of \(\text{CuCl}_2\cdot2\text{H}_2\text{O}\) (1 mmol, 0.170 g) with constant stirring at 70°C. The solution was refluxed for 10 h. A hot methanolic solution of 2,2'-bipyridine (1 mmol or 0.156 g) was added drop wise to above solution with refluxing continuing for further 8 h. Green precipitates were collected after filtration. Several washings were made with cold methanol. Yield: 55 %, Color: Green, M.P. Starts decomposing at 248°C, UV (\(\lambda_{\text{max}}\)): 292 nm, MS: \([\text{M}]^+ 558\), Main IR peaks (cm\(^{-1}\)): \(\nu(\text{OH}) 3200-3400\), \(\nu(\text{C}_6\text{H}_5\,\text{stretch}) 3051\), \(\nu(\text{C}=\text{N}) 1595\), \(\nu(\text{C}–\text{C}) 1510\), \(\nu(\text{M-N}) 416\).

2.3.6 Methodology for the synthesis of (gly-p-andn) Schiff base \((L_2)\)

To a stirred and refluxed solution of glyoxal (4 mmol, 0.232 g) in hot methanol (35 ml) was added drop wise a hot methanolic solution (35 ml) of \(p\)-anisidine (8 mmol, 0.985 g or 0.9 ml). The whole set up was placed into an oil bath for 5 h at 70°C. Yellow colored precipitates were formed immediately. The solution is further refluxed for 3 h to ensure complete precipitation. The precipitates thus obtained were filtered, washed many times with methanol. They were recrystallized using hot methanol as solvent. Yield: 80 %, Color: Yellow, M.P. 125°C, UV (\(\lambda_{\text{max}}\)): 236 nm, 373 nm, Main IR peaks (cm\(^{-1}\)): \(\nu(\text{OH}) 3300-3400\), \(\nu(\text{C}_6\text{H}_5\,\text{stretch}) 3062\), \(\nu(\text{C}=\text{N}) 1600\), \(\nu(\text{C}–\text{C}) 1583\). \(^1\)H NMR (400 MHz, \(d^6\) - DMSO) \(\delta = 8.39\,(s, 2\text{H, -CH=N}), 7.36\,(d, 4\text{H, Ar-H}), 6.95\,(d, 4\text{H, Ar-H}), 3.79\,(s, 6\text{H, -OCH}_3)\).

\[
\text{H}_2\text{N}\underbrace{\begin{array}{c} \text{OCH}_3 \\
\text{p-anisidine} 
\end{array}}_{\text{Glyoxal}} + \underbrace{\begin{array}{c} \text{methanol} \\
\text{reflux} 
\end{array}}_{\text{Fig. 3: Scheme for synthesis of Schiff base (L}_2)\text{)}
\]

2.3.7 Methodology for the synthesis of \([\text{Zn}(L_2)\text{phen}]\text{Cl}_2\) (5)

In hot methanolic (30ml) of Schiff base (1mmol, 0.268g) \((L_2)\) was added methanolic solution (30 ml) of \(\text{ZnCl}_2\) (1 mmol, 0.136 g) with constant stirring at 70°C. The solution was refluxed for 10 h. A hot methanolic solution of 1,10-phenanthroline (1 mmol or 0.198 g) was added drop wise to above solution with refluxing continuing for further 8 h. White
precipitates were collected after filtration. Several washings were made with cold methanol. Yield: 63 %, Color: White, M.P. Above 280°C, UV (λmax): 223 nm, 267 nm, MS: [M]+ 566, Main IR peaks (cm⁻¹): \( \nu(C_6H_5 \text{ stretch}) \) 3049, \( \nu(C=\text{N}) \) 1583, \( \nu(C-C) \) 1512, \( \nu(M-N) \) 405.

2.3.8 Methodology for the synthesis of [Zn(L2)bpy]Cl₂ (6)

In hot methanolic (30ml) of Schiff base (1mmol, 0.268g) (L₂) was added methanolic solution (30 ml) of ZnCl₂ (1 mmol, 0.136 g) with constant stirring at 70°C. The solution was refluxed for 10 h. A hot methanolic solution of 2,2'-bipyridine (1 mmol or 0.156 g) was added drop wise to above solution with refluxing continuing for further 8 h. White precipitates were collected after filtration. Several washings were made with cold methanol. Yield: 62 %, Color: White, M.P. Above 280°C, UV (λmax): 222 nm, 267 nm, MS: [M]+ 542, Main IR peaks (cm⁻¹): \( \nu(C_6H_5 \text{ stretch}) \) 3064, \( \nu(C=\text{N}) \) 1589, \( \nu(C-C) \) 1491, \( \nu(M-N) \) 414.

2.3.9 Methodology for the synthesis of [Cu(L2)phen]Cl₂ (7)

In hot methanolic (30 ml) of Schiff base (1 mmol, 0.268 g) (L₂) was added methanolic solution (30 ml) of CuCl₂·2H₂O (1 mmol, 0.170 g) with constant stirring at 70°C. The solution was refluxed for 10 h. A hot methanolic solution of 1,10-phenanthroline (1 mmol or 0.198 g) was added drop wise to above solution with refluxing continuing for further 8 h. Green precipitates were collected after filtration. Several washings were made with cold methanol. Yield: 55 %, Color: Green, M.P. Starts decomposing at 264°C, UV (λmax): 296 nm, MS: [M]+ 600, Main IR peaks (cm⁻¹): \( \nu(C_6H_5 \text{ stretch}) \) 3041, \( \nu(C=\text{N}) \) 1577, \( \nu(C-C) \) 1514, \( \nu(M-N) \) 430.

2.3.10 Methodology for the synthesis of [Cu(L2)bpy]Cl₂ (8)

In hot methanolic (30ml) of Schiff base (1 mmol, 0.268 g) (L₂) was added methanolic solution (30 ml) of CuCl₂·2H₂O (1 mmol, 0.170 g) with constant stirring at 70°C. The solution was refluxed for 10 h. A hot methanolic solution of 2,2'-bipyridine (1 mmol or 0.156 g) was added drop wise to above solution with further refluxing for 8 h. Green precipitates were collected after filtration. Several washings were made with cold methanol. Yield: 54 %, Color: Green, M.P. Starts decomposing at 254°C, UV (λmax): 296 nm, MS: [M]+ 541, Main IR
peaks (cm\(^{-1}\)): \(\nu(\text{OH})\) 3200-3400, \(\nu(\text{C}_6\text{H}_5\ \text{stretch})\) 3034, \(\nu(\text{C} = \text{N})\) 1566, \(\nu(\text{C} - \text{C})\) 1491, \(\nu(\text{M-N})\) 416.

### 2.4 Results and discussions

The ligands and Cu\(^{2+}\) mixed ligand chelates appear as colored precipitates while Zn\(^{2+}\) complexes appear as white precipitates. All of them were found to be thermally stable and were non hygroscopic solids. They do not show any signs of decomposition in air and moisture even after months and were having fair solubility in water, DMSO, DMF and Tris buffer (pH 7.4).

#### 2.4.1 UV-vis analysis

The UV-vis absorption spectroscopic results of Schiff bases and metal complexes were recorded in the range of 200 - 800 nm at low concentrations using water as solvent. The bands observed indicates \(\pi\) to \(\pi^*\) transitions confirming binding of metal centers with Schiff base, 1,10-phenanthroline / 2,2'-bipyridine. The UV–vis spectral peaks of the ligands and their chelates have been shown in Table 1. The low energy band in \(L_1\) and \(L_2\) at 239 nm and 236 nm respectively was assigned to \(\pi \rightarrow \pi^*\) transition of the aromatic ring while the second low energy band at 284 nm for \(L_1\) and at 285 nm for \(L_2\) was assigned to intermediate energy \(\pi \rightarrow \pi^*\) transitions of the \(-\text{CH}=\text{N}-\) group. The bands obtained in the range of 221- 296 nm for all the complexes were assigned to intra ligand charge transfer (\(\pi \rightarrow \pi^*\)) transitions. These bands suggest the presence of benzene ring in the ligand and complex moieties.

#### 2.4.2 FTIR analysis

In the uncoordinated ligand a strong band appears at 1599 cm\(^{-1}\) for \(L_1\) and 1600 cm\(^{-1}\) for \(L_2\) attributing to free azomethine group, but a negative shift up to 1566 cm\(^{-1}\) in metal chelates proposes coordination of the imine nitrogen to metal centers. This may occurs due to decrease in bond strength of imine bond and simultaneous increase in bond strength between azomethine nitrogen and metal centre. All the metal complexes show absorption peaks at 414 - 429 cm\(^{-1}\) region corresponding to M-N vibrations confirming the bond formation between azomethine nitrogen and metal ion. Absorption bands at 3200 - 3400 cm\(^{-1}\) range in some complexes marks the existence of coordinated or lattice water. IR analysis with selected bond...
frequencies of all Schiff bases and their corresponding mixed ligand chelates are as follows [35] (Table14):

Table 1: Selected bond frequencies (cm\(^{-1}\)) and UV-vis values of ligand and Zn(II) and Cu(II) mixed ligand chelates

| Complex                  | \(\nu_{(\text{M-N})}\) (cm\(^{-1}\)) | \(\nu_{(\text{C=H})}\) stretch (cm\(^{-1}\)) | \(\nu_{(-C=N-)}\) stretch (cm\(^{-1}\)) | Lattice water | \(\pi\) to \(\pi^*\) transition (nm) |
|--------------------------|--------------------------------------|---------------------------------------------|----------------------------------------|---------------|---------------------------------------|
| (L\(_1\))                | -                                    | 3045                                       | 1599                                   | -             | 239, 284                              |
| [Zn(L\(_1\))phen]Cl\(_2\) (1) | 426                                  | 3047                                       | 1581                                   | 3200-3400     | 221, 265                              |
| [Zn(L\(_1\))bpy]Cl\(_2\) (2)   | 412                                  | 3061                                       | 1595                                   | 3200-3400     | 239, 291                              |
| [Cu(L\(_1\))phen]Cl\(_2\) (3)   | 428                                  | 3048                                       | 1581                                   | 3200-3400     | 269                                   |
| [Cu(L\(_1\))bpy]Cl\(_2\) (4)   | 416                                  | 3051                                       | 1595                                   | 3200-3400     | 292                                   |
| (L\(_2\))                | -                                    | 3062                                       | 1600                                   | 3300-3400     | 236, 285                              |
| [Zn(L\(_2\))phen]Cl\(_2\) (5) | 405                                  | 3049                                       | 1583                                   | -             | 223, 267                              |
| [Zn(L\(_2\))bpy]Cl\(_2\) (6)   | 414                                  | 3064                                       | 1589                                   | -             | 222, 267                              |
| [Cu(L\(_2\))phen]Cl\(_2\) (7)   | 430                                  | 3041                                       | 1577                                   | -             | 296                                   |
| [Cu(L\(_2\))bpy]Cl\(_2\) (8)   | 416                                  | 3034                                       | 1566                                   | 3200-3400     | 296                                   |

2.4.3 Mass spectral analysis

The molecular ion peaks in mass spectra of ligands (L\(_1\) and L\(_2\)) was observed at m/z 269 which is the parent ion peak of both ortho and para isomers, thus confirming there formation. The mass fragmentation pattern of the complexes has been tabulated as follows:
| Complex                        | Fragmented ion                  | m/z |
|-------------------------------|---------------------------------|-----|
| [Zn(L₁)phen]Cl₂ (1)           | [Zn(L₁)phen]Cl₂.2H₂O           | 620 |
| - H₂O                         | [Zn(L₁)phen]Cl₂.2H₂O           | 602 |
| - L₁, - Cl                    | [Zn(phen)₂Cl]                  | 460 |
| - Cl                          | [Zn(phen)Cl]                   | 281 |
| - Zn                          | [Zn(phen)]                     | 245 |
| Free phen                     |                                 | 180 |
| [Zn(L₁)bpy]Cl₂ (2)            | [Zn(L₁)bpy]Cl₂.H₂O             | 578 |
| - L₁,- Cl,- H₂O               | [Zn(bpy)Cl]                    | 256 |
| - Cl                          | [Zn(bpy)]                      | 223 |
| - Zn                          | Free bpy                       | 157 |
| [Cu(L₁)phen]Cl₂ (3)           | [Cu(L₁)phen]Cl₂.H₂O            | 602 |
| - L₁,- Cl,- H₂O               | [Cu(phen)Cl]                   | 279 |
| - Cl                          | [Cu(phen)]                     | 243 |
| - Cu                          | Free phen                      | 180 |
| [Cu(L₁)bpy]Cl₂ (4)            | [Cu(L₁)bpy]Cl₂                 | 558 (Not recorded) |
| - bpy,- Cl₂                   | [Cu(L₁)]                       | 332 |
| - Cl                          | [Cu(bpy)Cl]                    | 255 |
| - Cu                          | [Cu bpy]                       | 219 |
| Free bpy                      |                                 | 157 |
| [Zn(L₂)phen]Cl₂ (5)           | [Zn(L₂)phen]Cl₂.H₂O            | 566 |
| - L₂                          | [Zn(phen)Cl]                   | 280 |
| - Zn,- Cl                     | Free phen                      | 180 |
| [Zn(L₂)bpy]Cl₂ (6)            | [Cu(L₂)bpy]Cl₂.H₂O             | 542 |
| - L₂                          | [Cu(bpy)Cl]                    | 256 |
| - Cu,- Cl                     | Free bpy                       | 157 |
[Cu(L₂)phen]Cl₂ (7)          [Cu(L₂)phen]Cl₂.H₂O  600  
- L₂, - Cl               [Cu(phen)₂Cl]  460  
- Cl                    [Cu(phen)]₂  423  
- phen                  [Cu(phen)Cl]  280  
- Cl                    [Cu(phen)]  243  
- Cu                    Free phen  180  

[Cu(L₂)bpy]Cl₂ (8)          [Cu(L₂)bpy]Cl₂.H₂O  541  
- bpy                   [Cu(L₂)]  331  
- L₂                    [Cu(bpy)Cl]  255  
- Cu, - Cl              Free bpy  157  

2.4.4 ¹H NMR spectrum analysis

The ¹H NMR of the L₁ was recorded in chloroform while that of L₂ was recorded in d⁶-DMSO. TMS was used as internal reference. A signal at 8.1 - 8.4 ppm in ligand spectra was due to azomethine protons. The multiplet in the range of 6.9 - 7.6 was assigned to protons of aromatic protons of benzene rings while a singlet at 3.7 - 3.8 was due to methoxy group. The ¹H NMR spectra of the Schiff bases is as follows:

¹H NMR of L₁: (400 MHz, CDCl₃), δ = 8.15 (s, 2H, -CH=N), 7.66 (d, 4H, Ar-H), 7.53 (d, 4H, Ar-H), 3.85 (s, 6H, -OCH₃)

¹H NMR of L₂: (400 MHz, d⁶-DMSO) δ = 8.39 (s, 2H, -CH=N), 7.36 (d, 4H, Ar-H), 6.95 (d, 4H, Ar-H), 3.79 (s, 6H, -OCH₃).

2.4.5 UV-vis absorption studies of BSA

UV-vis absorption spectroscopy acts as quite handy and reliable technique to scrutinize the interactive behaviour and structural changes of metal complexes with serum albumins. Firstly the solution of tris buffer (0.1 M) was prepared using doubly distilled water. Then BSA solution of 1000 μM concentration and metal complexes of 50 μM concentrations were prepared using tris buffer as solvent. The UV-vis spectra were recorded by taking static concentration of metal complex (50 μM) vs. dynamic [BSA] concentrations in the array of 0 -
There is a direct proportional relationship between the concentration of [BSA] and the intensity of band. Then their binding constants were calculated:

- By UV-vis titration graphs of metal complexes (50 μM) with incremental [BSA] concentration in the range of 0 - 3 μM,
- By plotting graph of BSA complex with metal chelates subtracting corresponding signal for different concentrations of [BSA],
- By plotting graph of 1 / [BSA] (on X - axis) vs. 1 / (A-A0) (on Y - axis) concentration, where A is the absorption signal of bounded complex at variant complex – [BSA] concentrations while A0 is an absorption signal of unbound complex.

Then the values of binding constants for each metal complex were determined. The interaction between substrate (S) and BSA concentration (L) is presumed to be of ratio 1:1, resulting into formation of a single complex (SL).

The relationship amid the pragmatic absorbance (cm⁻¹) alteration, different parameters and system variables can be calculated as follows:

\[
\Delta A = \frac{S_t K_{11} \Delta \varepsilon_{11} [L]}{1 + K_{11} [L]} \tag{1}
\]

where \( S_t \) is total concentration of substrate,

\( \Delta A = A - A_0 \),

\( \Delta \varepsilon_{11} = \varepsilon_{11} - \varepsilon_S - \varepsilon_L \)

Where \( \varepsilon_{11} \) signifies molar absorptivity of BSA - metal complex,

\( \varepsilon_S \) signifies molar absorptivity of unbound metal complex,

\( \varepsilon_L \) signifies molar absorptivity of the BSA.

From the mass balance expression \( S_t = [S] + [SL] \),
And \( [S] = S_t / (1 + K_{11} [L]) \).

Where \([S]\) signifies concentration of unbound metal complex,

\([L]\) signifies concentration of unbound BSA,

\([SL]\) signifies concentration of BSA – metal complex

Equation (1) shows that there is hyperbolic dependence on the concentration of unbound BSA, thus it signifies binding isotherms.

Therefore double reciprocal plot of 1 / (A-A0) vs. 1 / [BSA] comes out to be linear and the values of binding constant (\( K_b \) in M⁻¹) can be calculated as:
\[ K_b = \frac{\text{Intercept}}{\text{Slope}} \]

where \( A \): absorption signal at variant complex - [BSA] concentrations

\( A_0 \): absorption signal of unbound metal chelate [36-40].

**Table 2: Values of Binding constant values (\( K_b \ \text{M}^{-1} \))**

| Complex               | \( K_b \ \text{M}^{-1} \) |
|-----------------------|----------------------------|
| [Zn(L₁phen)Cl₂] (1)   | \( 4.46 \times 10^6 \)    |
| [Zn(L₁bpy)Cl₂] (2)    | -                          |
| [Cu(L₁phen)Cl₂] (3)   | -                          |
| [Cu(L₁bpy)Cl₂] (4)    | \( 6.9 \times 10^6 \)     |
| [Zn(L₂phen)Cl₂] (5)   | -                          |
| [Zn(L₂bpy)Cl₂] (6)    | \( 1.20 \times 10^5 \)    |
| [Cu(L₂phen)Cl₂] (7)   | \( 1.08 \times 10^6 \)    |
| [Cu(L₂bpy)Cl₂] (8)    | \( 4.18 \times 10^6 \)    |
Fig. 4: UV-vis titration curves of (a) [Zn(L₁)phen]Cl₂ complex (50 μM) with incremental [BSA] concentration in the range of 0 – 3 μM, (b) [BSA complex with [Zn(L₁)phen]Cl₂] – [Variant concentrations of [BSA]], (c) Graph of 1 / (A-A₀) vs. 1 / [BSA] concentration.
Fig. 5: (a) UV-vis titration graphs of (a) [Cu(L1)bpy]Cl2 complex (50 μM) with incremental [BSA] concentration in the range of 0 – 3μM, (b) Graph of \([\text{BSA complex with } [\text{Cu}(L1)\text{bpy}]\text{Cl2}] - \text{[Variant concentrations of [BSA]}\), (c) Graph of \(1 / (A-A0)\) vs. \(1 / \text{[BSA]}\) concentration.
Fig. 6: UV-vis titration graphs of (a) \([\text{Zn}(L_2)\text{bpy}]\text{Cl}_2\) complex (50 μM) with incremental [BSA] concentration in the range of 0 – 3μM, (b) Graph of \([\text{BSA complex with [Zn(L_2)\text{bpy}]Cl}_2] – [\text{Variant concentrations of [BSA]}]\), (c) Graph of \(1/(A - A_0)\) vs. \(1/[\text{BSA}]\) concentration.
Fig. 7: UV-vis titration graphs of (a) [Cu(L₂phen)]Cl₂ complex (50 μM) with incremental [BSA] concentration in the range of 0 – 3μM, (b) Graph of $\{[\text{BSA complex with } [\text{Cu}(L_2\text{phen})\text{Cl}_2] – [\text{Variant concentrations of } [\text{BSA}]}\}$, (c) Graph of $1/(A-A_0)$ vs. $1/[\text{BSA}]$ concentration.
Fig. 8: UV-vis titration graphs of (a) [Cu(L2)bpy]Cl2 complex (50 μM) with incremental [BSA] concentration in the range of 0 – 3μM, (b) Graph of {[BSA complex with [Cu(L2)bpy]Cl2 – [Variant concentrations of [BSA]}], (c) Graph of 1 / (A-A0) vs. 1 / [BSA] concentration
2.5 Antimicrobial Assays

Agar well diffusion process is used to evaluate the antimicrobial behaviour of ligands along with their metal chelates. The concentrations of the solutions were made by dissolving 5 mg of sample in 1 ml of solvent (DMSO). The bacterial and fungal cultures were homogeneously applied with sterile cotton swabs on mueller hinton agar (MHA) and potato dextrose agar (PDA) respectively. Sterilized cork borer of 7mm diameter was employed for cutting of wells in agar plates. Micropipette was used to add 100 μl of each sample to the wells. The plates were placed in a sterile place for 24 hours in case of bacteria and 48 hours for checking fungal growth. DMSO was used as negative control in well diffusion method. Antimicrobial activity of standard antibiotic drug amikacin against test bacteria and fluconazole against test fungi was evaluated by agar disc diffusion method [41]. Zone of inhibition surrounding each well / disc was measured to determine the antimicrobial activities. Each experiment was performed three times to minimize the deviations (Table 3).

Test Organisms: A) Bacteria: *Escherichia coli* and *Staphylococcus aureus*
B) Fungi: *Aspergillus niger* and *Aspergillus fumigatus*

Table 3: Antimicrobial activity of ligand and complexes (Concentration of 5 mg ml⁻¹)

| Complex | Average Inhibition Zone in diameter (mm) ± SD | Antibacterial Activity | Antifungal Activity |
|---------|---------------------------------------------|------------------------|---------------------|
|         |                                             |                        |                     |
|         |                                             | **E. coli** (A)         | **S. aureus** (B)   |
|         |                                             | **A. niger** (C)        | **A. fumigatus** (D) |
| (L₁)    |                                             | 10.33±0.29             | -                   |
| [Zn(L₁)phen]Cl₂ (1) | 16.66±0.58 | 35.16±0.29 | - |
| [Zn(L₁)bpy]Cl₂ (2)       | 11.00±0.58 | 25.33±0.58 | 22.50±0.50 | - |
| [Cu(L₁)phen]Cl₂ (3)       | 18.33±0.58 | 28.50±0.50 | 38.16±0.29 | 30.33±0.29 |
| [Cu(L₁)bpy]Cl₂ (4)       | 17.66±0.58 | 21.50±0.50 | 10.16±0.29 | - |
| (L₂)    | 10.66±0.58 | -         | 11.50±0.29 | - |
\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{Complex} & \text{Activity} & \text{Activity} & \text{Activity} & \\
\hline
[Zn(L_2)\text{phen}]Cl_2 (5) & 10.33\pm0.58 & 15.33\pm0.58 & 37.30\pm0.57 & - \\
\hline
[Zn(L_2)bpy]Cl_2 (6) & 12.66\pm0.58 & - & - & - \\
\hline
[Cu(L_2)\text{phen}]Cl_2 (7) & 22.00\pm0.36 & 30.40\pm0.36 & 38.16\pm0.29 & 33.50\pm0.50 \\
\hline
[Cu(L_2)bpy]Cl_2 (8) & 14.00\pm0.50 & 15.33\pm0.29 & 18.50\pm0.50 & 21.50\pm0.50 \\
\hline
\text{Amikacin} & 21.50\pm0.50 & 24.83\pm0.76 & - & - \\
\hline
\text{Fluconazole} & - & - & 23.66\pm0.57 & 22.66\pm0.29 \\
\hline
\text{DMSO} & \text{Nil} & \text{Nil} & \text{Nil} & \text{Nil} \\
\hline
\end{array}
\]

where SD is Standard Deviation

Fig. 9: Antimicrobial assay of Schiff base ligand (L_1) and its metal chelate (1 - 4) (Alphabet levels are according to table 16)

Fig. 10: Antimicrobial assay of Schiff base ligand (L_2) and its metal chelate (5 - 8) (Alphabet levels are according to table 16)

2.6 Conclusion

This work details about the synthesis of two Schiff bases (L_1) and (L_2) as primary ligand and their mixed ligand complexes with zinc(II) and copper(II) metal ions and 1,10-
phenanthroline or 2,2'-bipyridine as the secondary ligand. The reactants for the synthesis of ligands are selected as dialdehydes with different isomers of primary amine instead of conventional method of reaction between diamines and aldehyde or ketone. Schiff base of para isomer of primary amine precipitates readily while ortho isomer precipitates in a longer time span. The reaction with meta isomer resulted in a tar and the product could not be isolated despite several attempts. Both the ligands appear as bidentate, coordinating to metal centers through imine nitrogen. Absence of peaks in the 500 cm⁻¹ suggests the absence of M-O bond. Molecular mass of ligand and metal complexes are in consistence with their mass spectral data. Octahedral geometry has been proposed for all the complexes on the basis of evidences shown by their spectral studies with primary and secondary ligands at their equatorial position while axial positions may be occupied by weak coordinating anions or water molecules. Serum protein interactions of complexes were studied by determining the values of binding constants using UV-vis titration technique. The complexes show binding constants in the range of 10⁴ - 10⁵ M⁻¹. It is worth mentioning that moderate values of binding constants further strengthen the task of serum proteins in drug delivery at targeted sites as carrier molecules. In antimicrobial assays, pure ligands show least activity while metal complexes show moderate to high activity as compared to standard drug. The zone of inhibition with *Staphylococcus aureus* and *Aspergillus fumigatus* is the highest. The better activities of metal complexes as compared to ligands could be due to the manipulation of the hydrophobicity of the metal complexes over the hydrated metal ions by different ligands. This change in polarity of metal on coordination with the ligand may result in easy penetration of the complexes through the cell membrane of the microbes.

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2.8 Annexure

Annexure 2a: UV-vis spectra of (L₁) Schiff base and its metal complexes
Annexure 2b: UV-vis spectra of (L₂) Schiff base and its metal complexes
Annexure 2c: IR of (L₁)

Annexure 2d: IR of [Zn(L₁)phen]Cl₂

Annexure 2e: IR of [Zn(L₁)bpy]Cl₂

Annexure 2f: IR of [Cu(L₁)phen]Cl₂

Annexure 2g: IR of [Cu(L₁)bpy]Cl₂
Annexure 2h: IR of (L₂)

Annexure 2i: IR of [Zn(L₂)phen]Cl₂
Annexure 2j: IR of [Zn(L₂)bpy]Cl₂

Annexure 2k: IR of [Cu(L₂)phen]Cl₂
Annexure 2l: IR of [Cu(L₂)bpy]Cl₂
Annexure 2m: NMR of (L₁)

Annexure 2n: NMR of (L₂)
Annexure 2o: Mass spectra of L1 (Mol. Mass = 268 g)

Annexure 2p: Mass spectra of [Zn(L1)phen]Cl2 (I)
Annexure 2q: Mass spectra of [Zn(L₁)bpy]Cl₂ (2)

Annexure 2r: Mass spectra of [Cu(L₁)phen]Cl₂ (3)
Annexure 2s: Mass spectra of [Cu(L1)bpy]Cl2 (4)

Annexure 2t: Mass spectra of L2 (Mol. Mass = 268 g)
Annexure 2u: Mass spectra of [Zn(L_2)phen]Cl_2 (5)

Annexure 2v: Mass spectra of [Zn(L_2)bpy]Cl_2 (6)
Annexure 2w: Mass spectra of [Cu(L₂)phen]Cl₂ (7)

Annexure 2x: Mass spectra of [Cu(L₂)bpy]Cl₂ (8)