Pharmacologically significant tetraaza macrocyclic metal complexes derived from isatin and 3,4-diaminobenzophenone: Synthesis, spectral studies and comparative in-vitro biological assessment
4.1 INTRODUCTION

During the past decades, macrocyclic complexes of Schiff bases have been emerging as a focused area of research interest attracting attention of both inorganic and bioinorganic chemists globally. An important class of ligands consists of Schiff bases derived from the condensation of carbonyl and an amino group containing compounds that coordinate to metal ions through azomethine nitrogen. These Schiff bases ligands possess a broad spectrum of pharmacological activities such as antitumor, antibacterial, antifungal and anticancer activities. The macrocyclic ligand formation incriminates metal ion template to orient the reactant groups of linear substrate in the desirable confirmation for the ring closure rather than oligomeric or polymeric product. The unfavorable entropy overcome by favorable enthalpy for the formation of metal ligand bonds (M-L) ordering the multidentate ligand around metal ion and thus, promoting the cyclization reaction. In general, macrocyclic complex formation depends on the size and rigidity of the internal cavity of the macrocycle. The metal complexes of Schiff bases obtained from heterocyclic moieties comprising of S, N and/or O as ligand atoms are of great importance as simple structural biological models. Hence, biological properties of carbocycles (a ring composed exclusively of carbon atoms) are reduced rather than macrocyclic complexes consisting of heterocyclic moieties in the macrocyclic rings in which macrocyclic framework having pendant arms can enhance the selectivity of the ligand for a given ion allowing fine-tuning of the metal complexes properties. Isatin (1H-indole-2,3-dione), is a privileged scaffold and among the most promising class of heterocyclic compound that possesses many interesting activity profiles and well-tolerated in humans which have acquired significant applications in medicinal chemistry because of their wide spectrum of biological activities. There have been many studies involving various metal complexes of Schiff base macrocycles derived from isatin derivatives. In view of their pharmacological and biological properties, the isatin based Schiff base macrocyclic compounds opened up a new vista of promising prospects in synthetic organic chemistry. The antibacterial and antioxidant parameters are vital in the prevention of human diseases. Antioxidant compounds may act as free radical scavengers, playing crucial role in degradation of food and chemical materials, considerably preventing the oxidation of readily oxidable substances. The formation of high levels of reactive oxygen species (ROS) including hydrogen peroxide,
superoxide anion and hydroxyl radical resulting from various physiological or biochemical processes or by pollution and other exogenous sources, can be harmful to the body and may damage all the major components of cells (DNA, proteins, and cell membranes) leading to several diseases, such as arthritis, inflammation, ageing, cancer, cardiovascular, pulmonary and neurodegenerative disorders.\textsuperscript{14,15} The free radical formation in different diseases has developed the curiosity for the exploration of compounds which are termed as antioxidants that resist the formation of free-radicals or compounds which may act as oxygen radical scavenger.\textsuperscript{16} Isatin-based drug candidates have already been pursued in the pharmaceutical and medicinal industries. For example, the multiple tyrosine kinase receptor inhibitors Semaxanib and TSU-68 were discovered as the emerging class of compounds among class of small-molecule inhibitors to develop potential anticancer treatment.\textsuperscript{17} Structurally, Semaxanib and TSU-68 are close analogs of Sunitinib (orally administered tyrosine kinase inhibitor approved for the treatment of the metastatic renal cell carcinoma) as it contain isatin motif.\textsuperscript{18} Recently, the advancement in antibiotic resistant microorganism has led to an increase in demand for new antibiotics.\textsuperscript{19} The biofilms are microbial communities which are coupled to the surface and often embedded in a hydrated matrix of polysaccharide and protein.\textsuperscript{20} Biofilm formation increases the resistance to antimicrobial agents that restrict the eradication of microorganism (the bacteria within biofilms are 10-1000 times more resistant to antibiotics and chemical agents).\textsuperscript{21-23} The biofilm formation is common in foodstuff and marine industry along with medical sector (up to 60% of the total human infections are provoked by biofilms).\textsuperscript{24-26} The Candida biofilms formation has important clinical application on the grounds of increased resistance to antifungal therapy and its ability to withstand host immune defenses. Among this division, \textit{Candida albicans} is the major species inducing life-threatenning infections among immunocompromised individuals like HIV and patients relying on distinct variety of implants. Therefore, there is an alarming call to search alternatives to the conventional drugs so as to encounter \textit{C. albicans} biofilm.\textsuperscript{27}

DNA is an important drug target, regulating various biochemical processes that occur in the biological system.\textsuperscript{28} Cisplatin (cis-diaminedichloroplatinum (II) or cis-[PtCl\textsubscript{2}(NH\textsubscript{3})\textsubscript{2}]) is the most potent antitumor drug available in the cancer chemotherapy of solid tumors such as lung, ovarian, head, neck and bladder cancers. Cisplatin promotes cancer cell death through binding with DNA.\textsuperscript{29} However, cisplatin
is nephrotoxicity, show poor water solubility, exhibit intrinsic resistance and covalent mode of their interaction with DNA which bound its clinical efficacy.\textsuperscript{30} Hence, considerable attention is focused to design new metal complexes as anticancer drugs which involve non-covalent modes of interaction with DNA.\textsuperscript{31} The interaction of transition metal complexes with DNA has been extensively studied during the last decade because many compounds expose their antitumor activity through binding with DNA and can cause DNA damage of cancer cells by blocking the cell division that results in cell death.\textsuperscript{32} The Schiff bases have drawn considerable application in the field of bioinorganic because of their structural variability, stability and biocompatibility.\textsuperscript{33}

Considering the earlier report which demonstrated that both the carbonyl group of isatin reacts with amine group,\textsuperscript{34} an effort has been made to synthesize pharmacologically active 12-membered tetraaza schiff base macrocyclic complexes comprising of isatin and 3,4-diaminobenzophenone via template condensation reaction for the metal series of Co(II), Ni(II), Cu(II) and Zn(II) and characterized via various physicochemical and spectroscopic techniques. To investigate the binding mode of complexes with DNA, fluorescence and circular dichroism study were performed. The molecular docking studies were undertaken for complex 3 and 4. Furthermore, the pharmacological efficacy of the newly synthesized complexes was explored by examining their antioxidant, antimicrobial, antibiofilm activities.

\subsection*{4.2 EXPERIMENTAL}

\subsubsection*{4.2.1 Materials and methods}
Isatin, 3,4-diaminobenzophenone, EB [Ethidium Bromide], CT-DNA [Calf Thymus], DPPH [2, 2-diphenyl-1-picrylhydrazyl], ascorbic acid and XTT [(2, 3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5 Carboxanilide)] and RPMI 1640 were procured from Sigma-Aldrich, USA. All the chemicals and solvents were used as supplied without further purification. The metal salts, MCl\textsubscript{2}.nH\textsubscript{2}O [M = Co, n = 6; M = Ni, n = 6; M = Cu, n = 2; M = Zn (Aldrich)] were commercially available pure samples. Microanalytical data (C, H and N) were obtained from Flash 2000 CHN elemental analyzer at CIL-Chandigarh, India. NMR spectra were recorded in DMSO-d\textsubscript{6} using a Bruker Avance II 400 NMR Spectrometer. The FT-IR (4000-400 cm\textsuperscript{-1}) was recorded
on FTIR; Interspec 2020, spectrolab U.K. using as KBr disc. ESI mass spectra were certified on a Q-ToF (Water Company) mass spectrometer. The electronic spectra (10⁻³ M, range 200-800 nm) of the complexes in DMSO were performed on Perkin Elmer λ-25 spectrophotometer at room temperature. The EPR spectra for Cu(II) and Co(II) complexes were recorded on JEOL JES-FA200 electron spin resonance (ESR) at 298 K and ES-DVT4 Spectrometer at liquid nitrogen temperature (LNT), respectively. The evaluation of magnetic susceptibility was carried out using a Faraday balance at room temperature. The molar conductivity data (10⁻³, mol L⁻¹) in DMSO solutions were recorded on a Systronic type 302 conductivity bridge equilibrated at 25 °C ± 0.01. To analyze thermal stability of complexes TGA/DTA study was performed on a Shimadzu DTG-60H system (Japan) under N₂ flow (20 mL min⁻¹) with heating rate of 10 °C/min. The sample morphology were recorded onto carbon tapping copper stubs and before SEM observations, the samples were coated with gold in the JEOL-JFC-1600 auto fine coater for 50 s at 20 mA and then coated samples were mounted into a sample holder for the SEM analysis at different magnifications. The in-vitro antimicrobial assessment of the compounds was performed using both disk diffusion and broth micro dilution methods. The antibiofilm potential of synthesized compound (complex 3) was also determined by using XTT assay. The free radical scavenging property of the Schiff base macrocyclic complexes is commonly evaluated using DPPH method.

4.2.2 Synthesis of Schiff base tetraaza macrocyclic complexes: Dichloro[5,6;11,12-dibenzophenone-1,4,7,10-tetraazacyclododeca-1,3,7,9-tetraene-2,3,8,9-diindole] metal(II), [MLCl₂] where M = Co(II), Ni(II), Cu(II) and Zn(II)

All the complexes were synthesized by template method where methanolic solution (30 mL) of 3,4-diaminobenzophenone (0.42 g, 2 mmol) was treated with metal(II) chloride (1 mmol) dissolved in methanol (15 mL). The resulting dark color solution was refluxed on water bath for half an hour followed by the addition of isatin (0.29 g, 2 mmol) in methanol (30 mL). The refluxing was continued for 10-12 h and the resulting mixture was concentrated to half of its volume and cooled to room temperature and allowed to stand overnight. The precipitate thus formed was filtered, washed thoroughly with methanol and dried in vacuo over fused CaCl₂ (Scheme 4.1).

[Co(II)LCl₂] (1): Yield: 65%; Colour: chocolate. m.p.: 290 °C. Molar Conductance (1 × 10⁻³ M, DMSO): \( \Lambda_m \) (27 mol⁻¹ cm² Ω⁻¹). Anal. Calc. for C₄₂H₂₆N₆O₂Cl₂Co: C,
64.95; H, 3.37; N, 10.80; O, 4.12. Found: C, 64.82; H, 3.12; N, 10.69; O, 4.07%. IR (KBr, cm\(^{-1}\)): 1668 (C=O), 1604 (C=N), 3277 (N-H), 3030 (C-H), 452 (M-N). UV-vis (\(\lambda_{\text{max/nm}}\)): 688, 454, 351, 279. ESI-MS (m/z) = 776.53. Found: [M + H]\(^+\) = 777.26

[\textbf{Ni(II)LCl}, 2\textit{i}]: Yield: 47%; Colour: Brick red. m.p.: 275 °C. Molar Conductance (1 \times 10^{-3} M, DMSO): \(\lambda_m\) (28 mol\(^{-1}\) cm\(^2\) Ω\(^{-1}\)). Anal. Calc. for C\(_{42}\)H\(_{26}\)N\(_6\)O\(_2\)Cl\(_2\)Ni: C, 64.98; H, 3.37; N, 10.82; O, 4.12. Found: C, 64.87; H, 3.39; N, 10.72; O, 4.23%. IR (KBr, cm\(^{-1}\)): 1678 (C=O), 1615 (C=N), 3265 (N-H), 3062 (C-H), 490 (M-N). UV-vis (\(\lambda_{\text{max/nm}}\)): 715, 626, 336, 280. ESI-MS (m/z) = 776.29 Found: 776.91

[\textbf{Cu(II)LCl}, 3\textit{i}]: Yield: 61%; Colour: Cinnamon. m.p.: 250 °C. Molar Conductance (1 \times 10^{-3} M, DMSO): \(\lambda_m\) (23 mol\(^{-1}\) cm\(^2\) Ω\(^{-1}\)). Anal. Calc. for C\(_{42}\)H\(_{26}\)N\(_6\)O\(_2\)Cl\(_2\)Cu: C, 64.57; H, 3.35; N, 10.75; O, 4.09. Found: C, 64.49; H, 3.31; N, 10.61; O, 4.01%. IR (KBr, cm\(^{-1}\)): 1640 (C=O), 1595 (C=N), 3350 (N-H), 3080 (C-H), 510 (M-N). UV-vis (\(\lambda_{\text{max/nm}}\)): 561, 462, 335, 284. ESI-MS (m/z) = 781.14. Found: [M + H]\(^+\) = 782.08

[\textbf{Zn(II)LCl}, 4\textit{i}]: Yield: 59%; Colour: Rust. m.p.: 40 °C. Molar Conductance (1 \times 10^{-3} M, DMSO): \(\lambda_m\) (40 mol\(^{-1}\) cm\(^2\) Ω\(^{-1}\)). Anal. Calc. for C\(_{42}\)H\(_{26}\)N\(_6\)O\(_2\)Cl\(_2\)Zn: C, 64.42; H, 3.34; N, 10.73; O, 4.08. Found: C, 64.29; H, 3.11; N, 10.66; O, 4.03%. IR (KBr, cm\(^{-1}\)): 1650 (C=O), 1610 (C=N), 3335 (N-H), 3085 (C-H), 515 (M-N). \(^1\)H NMR (DMSO-\(d_6\), ppm): 12.10 (s, C-NH-C, 2H), 7.37-8.50 (m, 24H, Ar-H). \(^{13}\)C NMR (DMSO-\(d_6\), ppm): 195.52 (C=O), 143.07 and 137.44 (C=N), 111.56-133.81 (Ar-C). ESI-MS (m/z): 782.98. Found: [M + H]\(^+\) = 783.80
Scheme 4.1 Schematic illustration of Schiff base macrocyclic complexes of Co(II), Ni(II), Cu(II), and Zn(II).

4.3 CHARACTERIZATION

4.3.1 Solution preparation for DNA binding

A stock solution of the compounds was made by dissolving the complex in an aqueous solution of DMSO as the co-solvent and dilution was made with the corresponding phosphate buffer (pH = 7.2) to obtain desired concentrations. The final
DMSO concentration was kept below 0.3% v/v. The CT-DNA concentration was determined by the absorption intensity at 260 nm using the molar absorption coefficient value of 6600 M⁻¹cm⁻¹. The purity of CT-DNA solution was confirmed by UV absorbance ratio (A₂₆₀/A₂₈₀ = 1.9), indicating that CT-DNA is free from protein impurity.³⁷

### 4.3.2 Fluorescence spectroscopy

The fluorescence titration measurements were performed on spectrofluorophotometer model RF-5301 (Shimadzu Japan) at 25 ± 0.1 °C with a 1.00 cm optical path length cell, equipped with a 150 W Xenon lamp and both the excitation and emission slits were adjusted at 5 nm and 10 nm, respectively. The Intrinsic fluorescence was recorded by exciting the CT-DNA solution at 478 nm and emission spectra was carried out in 500-700 nm range. To evaluate the binding parameters, 1:1 ratio of DNA-EB (50 µM) complex solution was taken in quartz cell and the fluorimetric titrations were done with increasing concentration of tested compound (0-70 µM).

### 4.3.3 Circular Dichroism (CD) measurements

The secondary structural changes in CT-DNA were recorded on JASCO CD J-815 spectropolarimeter equipped with a Peltier-type temperature controller at 25 °C using Quartz cuvettes of 1 cm path length. Two scans were accumulated at a scan speed of 100 nm min⁻¹, with data being collected in the far-UV range of 230-320 nm under N₂ atmosphere. CD spectra of CT-DNA in absence and presence of compounds (0-20 µM) were also recorded and the respective blanks were subtracted from each spectrum.

### 4.3.4 Free radical scavenging activity

The free radical scavenging property of the synthesized Schiff base macrocyclic complexes were assessed with the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.³⁸ DMSO was used for the preparation of different concentrations of the test compounds (50-450 µg/mL). 3 mL of DPPH (0.1 mM) methanolic solution was added to the tubes containing 1 mL of sample solution of variable concentration and the resulting mixture were shaken vigorously and kept at ambient temperature. After 30 min incubation period, the absorbance of test solutions was measured at 517 nm. The experiment for the control was performed as mentioned before without the test
samples. The experiments were performed in duplicate. The DPPH scavenging ability of the compounds was determined by the following equation:

\[
\% \text{ RSA} = \left( \frac{A_0 - A_c}{A_0} \right) \times 100
\]

where RSA = radical scavenging activity, \( A_0 \) = absorbance of ascorbic acid (reference) and \( A_c \) = absorbance of test compounds at concentration, \( c \).

**4.3.5 In-vitro antimicrobial assay**

The bacterial strains (Escherichia coli, ATCC-25922; and Staphylococcus aureus, ATCC-25923) were inoculated into molten Nutrient Broth (NB) and incubated at 37 °C for 24 h while the fungal cells (Candida albicans, MTCC-183) were inoculated into Yeast Extract-Peptone-Dextrose Broth (YPD) at 24 °C for 48 h.

**4.3.5.1 Determination of antibacterial activity by agar diffusion assay**

The antibacterial activities of the complexes evaluated in terms zone of inhibition using disk diffusion method against E. coli as Gram-negative bacteria and S. aureus as Gram-positive bacteria while C. albicans was considered as a candidate for in-vitro antifungal assay. The pure cultures of microorganism were sub-cultured in broth at 35 °C ± 2 °C. A lawn of culture was adjusted for bacterial growth by spreading 100 µL fresh culture with \( 10^6 \) CFU/mL of test organism on nutrient agar plates using sterile glass rod. Afterwards, the plates were kept for suitable period of time to get absorbed. Finally, the wells (6 mm diameter) were punched into the seeded agar plates and loaded with 10 µL volume having concentration of 10 mg/mL of the tested compounds followed by incubation of plates at 37 °C for 24 h and subsequently, the diameter of inhibition zone around each disk was measured. The antibacterial activity of a common standard antibiotic Gentamicin and antifungal Amphotericin B was also recorded using the same procedure as above using the same concentration and solvents. Each experiment was done in triplicate to minimize the deviations.

\[
\% \text{ Activity Index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibiton by standard (diameter)}} \times 100
\]

**4.3.5.2 Determination of minimum inhibitory concentration assay**

The lowest concentration of an antimicrobial compound which inhibits the visible growth of microorganisms after 24 h of incubation period is termed as MIC. The MICs are decisive to assure the resistance of microorganisms to antimicrobial agents. The broth micro dilution assay was performed based on the method of NCCLS,
For the evaluation of MIC (µg/mL), the test compounds were taken in 5% DMSO (which has no activity against test microorganisms) to make 10 mg/mL of concentration to get final concentration of 1 mg/mL by proper dilution and the controls were inoculated with approximately ~10⁵ CFU/mL of actively dividing bacteria. The experiments were performed in duplicate. The bacterial culture plates were incubated for 18-24 h at 37 °C and the fungal strains were incubated at 24 °C for 24-48 h. The MICs for different test compounds were monitored visually and spectrophotometrically after incubation period.

**4.3.6 Interaction between bacteria and Schiff base complex: SEM analysis**

For SEM analysis of respective bacterial and fungal cells, a reported protocol was followed. Briefly, in 6 well cell culture plates, biofilm formation was initiated on sterile cover slip discs by distributing a standardized cell suspension (2 mL of a suspension containing 1 x 10⁶ cells/mL in RPMI 1640) onto proper discs at 37 °C for 2 h. Finally, the non adherent cells for biofilm formation were removed by washing. The cover slip discs were kept for incubation in 400 µL of RPMI media for another 24 h. Thereafter, the cover slips were loaded with 200 µg/mL (50 µL) of treated compound for 30 min. The discs were withdrawn and washed thrice completely in sterile PBS. The biofilms were fixed in 4% [vol/vol] formaldehyde and 1% [vol/vol] glutaraldehyde in PBS for 24 h. Then samples were rinsed thoroughly using 0.1 M PBS and subsequently in 1% osmium tetraoxide for 30 min. The samples were dehydrated afterwards through a series of ethanol washes and finally dried to observe under SEM.

**4.3.7 XTT biofilm assay**

The biofilms assay was performed using the polystyrene, sterile, 96-well microtiter plates. Following the incubation (2 h) of respective bacterial and fungal cell suspension (at the density of 10⁷ cells) in the wells at 37 °C, the plates were rinsed three times with PBS to remove loosely attached and un-adhered cells. Further, the plates were incubated with 100 µL per well of RPMI media for another 4 h at 37 °C to allow the biofilm development. After 4 h of incubation, when biofilms were still developing, the biofilms were given dark exposure of treated sample i.e., complex 3. Consequently, the plates were incubated at 37 °C for another 20 h. Finally, the sodium salt of XTT was dissolved in Phosphate buffer to 1 mg/mL concentration, filter-
sterilized and stored at -80 °C. Menadione was dissolved in acetone to 1 mM followed by immediate sterilization before each experiment. 100 µL of XTT/menadione solution was then added to each well of microtiter plate with gentle shaking using rotator incubator (100 rpm), then covered (in dark) and incubated at 37 °C for 5 h. The colorimetric change by water soluble formazan product was measured at 450 nm using a microplate reader (BioRad, USA). The wells without biofilms were taken as a blank.

4.3.8 Molecular docking
Molecular graphics program (Hex 8.0.0), was used to understand the interaction between synthesized metal complexes and DNA. Structure of the CT-DNA dodecamer d(CGCGAATTCGCG)₂ (PDB ID: 1BNA) was obtained from the protein data bank (http://www.rcsb.org./pdb). The metal complex files were drawn using ChemDraw (MM2) and their energies were minimized using Mercury software and then file was saved in PDB (PROTEIN DATA BANK) format. The water molecules were extracted from the 1BNA using chimera 1.10.2 before operating docking calculations. The extraction of water molecules from the 1BNA were done using chimera 1.10.2 before performing docking calculations.

4.4 RESULTS AND DISCUSSION
A novel series of 12-membered Schiff base macrocyclic complexes of the type [M(C₄H₈N₆O₂)Cl₂], [M = Co(II), Ni(II), Cu(II), Zn(II)] were derived from 3,4-diaminobenzophenone and isatin in methanolic medium using template condensation method (Scheme 4.1). The complexes were soluble in DMF and DMSO but insoluble in common organic solvents and water. The molar conductance values obtained for these complexes (in DMSO 10⁻³, mol L⁻¹) are in the range of 27-40 mol⁻¹ cm² Ω⁻¹ which indicated their non electrolytic nature.⁴² while the proposed stoichiometry is in accordance with the analytical results. The macrocyclic complexes thus formed were studied through various spectroscopic, physicochemical and biological characterizations.
4.4.1 FT-IR analysis

The bands expected in 3200-3400 cm\(^{-1}\) range, characteristic for \(\nu(\text{NH}_2)\) of 3,4-diaminobezophenone were absent in the FT-IR spectra of all the complexes. Furthermore, no characteristic absorption band of \(\nu(\text{C}=\text{O})\) group of isatin was observed (at 1735 cm\(^{-1}\)) that evident the formation of the macrocyclic framework from the condensation of carbonyl groups of isatin and amino groups of 3,4-diaminobenzophenone \((\text{Figure 4.1})\).

![Figure 4.1 FT-IR spectrum of Schiff base macrocyclic complex of Co(II), 1.](image)

A strong absorption band appeared in range of 1595-1615 cm\(^{-1}\) may be ascribed to \(\nu(\text{C} = \text{N})\) (azomethine group) vibrations while single medium intensity bands of N-H stretching vibrations of the isatin moiety appeared in the range of 3265-3350 cm\(^{-1}\) in all the complexes.\(^{33,34}\) However, an intense absorption band observed in the range of 1640-1678 cm\(^{-1}\) of the complexes may be assigned to pendant carbonyl group of 3,4-diaminobenzophenone.\(^{45}\) The absorption bands assigned for aromatic C-H stretching vibrations were noticed in the range of 3030-3085 cm\(^{-1}\). The absorption bands in the range of 1452-1477, 1045-1069 and 720-752 cm\(^{-1}\) may be accredited to phenyl ring vibrations. The C-N stretching vibrations may occur in the range of 1015-1355 cm\(^{-1}\). The far IR spectra of the complexes showed bands in the region 452-515 cm\(^{-1}\) corresponding to \(\nu(\text{M-N})\) stretching vibrations which give insight into the
coordination of the azomethine nitrogen to the central metal atom.\textsuperscript{46} The bands appeared in the range of 315-330 cm\textsuperscript{-1} were assigned to υ(M-Cl) stretching vibration.

4.4.2 \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectra

The \textsuperscript{1}H NMR spectrum of macrocyclic complex of Zn(II) showed a broad signal at 12.10 ppm, possibly assigned to the NH protons (s, 2H) of the isatin moiety\textsuperscript{47,48} [Figure 4.2(a)].

![Figure 4.2(a) \textsuperscript{1}H spectrum of Schiff base macrocyclic complex of Zn(II), 4.](image)

The multiplet appeared in the regions 7.37-8.50 ppm correspond to aromatic ring protons of isatin and 3,4-diaminobenzophenone moiety.\textsuperscript{49} The macrocyclic Schiff base complex fabrication has been validated by the absence of resonance signal expected at 6.24 ppm corresponding to primary amine (NH\textsubscript{2}) of 3,4-diaminobenzophenone.\textsuperscript{50}

The \textsuperscript{13}C-NMR spectrum of Zn(II) complex revealed strong resonance signal at 195.52 ppm assigned to pendant carbonyl carbons (>C=O) of 3,4-diaminobenzophenone moiety\textsuperscript{51} [Figure 4.2(b)]. The absence of signal at 184 and 159
ppm inferred complete condensation of isatin with 3,4-diaminobenzophenone while the resonance signals observed at 143.07 and 137.44 ppm belonged to characteristic imine functions (>C=N). Moreover, the presence of the signals in the range of 111.56-133.81 ppm suggested the aromatic carbons in the macrocyclic framework at their appropriate positions related to the proposed structure.

Figure 4.2(b) $^{13}$C NMR spectrum of Schiff base macrocyclic complex of Zn(II), 4.

4.4.3 Electro spray ionization mass spectra

The molecular ion peak of four complexes viz. 1, 2, 3, and 4 appeared at their respective values 777.26, 776.91, 782.08 and 783.80 (experimental). While the molecular ion peak for simulated mass spectra of complexes 1, 2, 3 and 4 appears at 776.53, 776.29, 781.14 and 782.98. The difference of mass could be attributed due to the presence of [M±H]$^+$ peaks. Also, the presence of peak for complex 1, 2, 3, and 4 at 778.12, 777.92, 783.09 and 784.01 respectively, correspond to the nitrogen isotope. The mass spectra of Schiff base macrocyclic complexes of Co(II), (a) Ni, (b) Cu(II), (c) and Zn(II), (d) have been displayed in Figure 4.3.
Figure 4.3 Mass spectra of Schiff base macrocyclic complexes of Co(II) (a), Ni(II) (b), Cu(II) (c) and Zn(II) (d).
4.4.4 Electronic spectral data

The UV-visible spectra of all the metal complexes displayed two bands in range of 270-365 nm represent $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions of aromatic rings and C=N groups, respectively\(^5\) (Figure 4.4).

![Figure 4.4 The solution state UV-vis spectra of Schiff base macrocyclic complexes of Co(II), (1); Ni(II), (2); and Cu(II), (3) in DMSO, $1.60 \times 10^{-3}$ M with path length 1 cm.](image)

The electronic spectrum of complex 1 exhibit two absorption bands at 454 and 688 nm, comparable to the characteristic features of octahedral Co(II) complex,\(^5\) and were reasonably assigned to $^4T_{1g}(F)\rightarrow^4T_{2g}(F)$ and $^4T_{1g}(F)\rightarrow^4A_{2g}(F)$ transitions, respectively. The observed magnetic moment of Co(II) complex was found to be 4.82 B.M at room temperature corresponding to three unpaired electrons, which is comparatively higher than spin-only value, i.e., 3.87 B.M for $d^7$ configuration. This deviation from the spin only value may be due to the spin-orbit coupling.\(^5\) The spectrum of complex 2 exhibited two absorption bands at 626 and 715 nm which may correspond to $^3A_{2g}(F)\rightarrow^3T_{1g}(F)$ and $^3A_{2g}(F)\rightarrow^3T_{1g}(P)$ transitions, respectively, characteristic of octahedral geometry around Ni(II) which is further corroborated by the value obtained for magnetic moment in case of Ni(II) i.e., 3.2 B.M.\(^5\) The distorted octahedral geometry around Cu(II) complex, 3 was evidenced by its electronic spectrum displaying the two absorption bands at 462 and 561 nm corresponding to $^2B_{1g}\rightarrow^2B_{2g}$ and $^2B_{1g}\rightarrow^2E_g$ transitions, respectively,\(^5\) in accordance with the observed magnetic moment values of 1.8 B.M.
4.5.5 EPR spectra of Cu(II) and Co(II) complexes

The EPR spectrum of Cu(II) complex, 3 was recorded on X band at frequency 9.3 GHz under the influence of magnetic field 3260 G at room temperature (300 K) [Figure 4.4 (a)].

![Figure 4.5(a) X band EPR spectrum of Cu(II) complex, (3) at room temperature (300 K)](image)

The spectrum did not exhibit any hyperfine splitting and displayed axial signal. The Cu(II) complex showed the signal at 2.09 and 2.04 for $g_{\parallel}$ and $g_{\perp}$, respectively. The observed trend of $g_{\parallel} > g_{\perp} > g_e$ (2.0023) for the present complex suggested that the unpaired electron is localized in $dx^2-y^2$ orbital of Cu(II) ion characteristic of the axial symmetry resulting into a tetragonally elongated structure of Cu(II) complex. The value of $g_{av}$ for the Cu(II) complex (2.05) was computed from $g_{av}^2 = g_{\parallel}^2 + 2g_{\perp}^2/3$ and its deviation from that of the free electron (2.0023) which may be explained in view of the covalent character in M-L bonding which was supported by Kivelson and Neiman. The greater value of $g_{\parallel}$ compared to $g_{\perp}$ indicated axially distorted octahedral geometry. According to Hathaway, the exchange interaction parameter, $G$, between Cu(II) centers is derived using the expression $G = (g_{\parallel} - 2.0023)/(g_{\perp} - 2.0023)$. As the value of $G < 4$, the considerable inter-exchange interaction between Cu(II) centers is expected in the solid state, as compared to $G > 4$ where inter-exchange interaction will be negligible in the solid complex. The
calculated G value (2.32) for the Cu(II) complex is less than 4 suggestive of copper-copper exchange interactions. Additionally, the observed $g_{II} > 2.30$ is indicative of an ionic environment and $g_{II} < 2.30$ represents that the complex has considerable covalent character in M-L bonding. The $g_{II}$ value for the present complex showed considerable covalent character.

The cobalt ion exhibits two oxidation states (II and III), where Co(III) with a $t^6_{2g}$ configuration is diamagnetic in nature and hence EPR silent. However, Co(II) exist in both low spin ($S = 1/2$) and high spin ($S = 3/2$) states and is paramagnetic, and so can be investigated by EPR spectroscopy. The EPR spectrum of the Co(II) complex was recorded in DMF solution at liquid nitrogen temperature (LNT) because the fast relaxation of Co(II) broaden the lines at higher temperature. The values of $g_{II}$ and $g_\perp$ observed to be 4.58 and 1.93, respectively [Figure 4.5 (b)]. The high deviation of the g values from the spin-only value i.e., 2.0023, is due to the large angular momentum contribution.

![EPR spectrum of Co(II) complex, (1) at 77 K.](image)

**Figure 4.5(b) EPR spectrum of Co(II) complex, (1) at 77 K.**

### 4.4.6 TGA/DTA analysis

The thermal stability of all the complexes represented the degradation of the schiff base metal complexes which started from 210 °C and ended at 800 °C (Figure 4.6, Table 4.1).
Figure 4.6 TGA/DTA curves of Schiff base macrocyclic complexes of Co(II), (1); Ni(II), (2); Cu(II), (3) and Zn(II), (4).
### Table 4.1 Stepwise thermal degradation data obtained from TGA/DTA curves of Schiff base macrocyclic complexes (1-4).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Decomposition Temp (°C)</th>
<th>% Weight loss</th>
<th>% Weight of residue</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex 1</td>
<td>230-450</td>
<td>56.04 (55.42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>450-700</td>
<td>37.38 (37.82)</td>
<td>6.54 (7.58)</td>
<td>Loss due to 2 coordinated chloride ion and benzophenone moiety</td>
</tr>
<tr>
<td></td>
<td>700-800</td>
<td></td>
<td></td>
<td>Loss due to remaining organic moiety</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Co residue</td>
</tr>
<tr>
<td>Complex 2</td>
<td>210-500</td>
<td>55.55 (55.44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500-690</td>
<td>37.03 (36.84)</td>
<td>7.40 (7.56)</td>
<td>Loss due to 2 coordinated chloride ion and benzophenone moiety</td>
</tr>
<tr>
<td></td>
<td>690-800</td>
<td></td>
<td></td>
<td>Loss due to remaining organic moiety</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ni residue</td>
</tr>
<tr>
<td>Complex 3</td>
<td>280-510</td>
<td>55.46 (55.10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>510-630</td>
<td>36.97 (36.65)</td>
<td>7.55 (8.13)</td>
<td>Loss due to 2 coordinated chloride ion and benzophenone moiety</td>
</tr>
<tr>
<td></td>
<td>630-800</td>
<td></td>
<td></td>
<td>Loss due to remaining organic moiety</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cu residue</td>
</tr>
<tr>
<td>Complex 4</td>
<td>270-510</td>
<td>54.99 (54.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>510-640</td>
<td>36.17 (36.56)</td>
<td>8.82 (8.35)</td>
<td>Loss due to 2 coordinated chloride ion and benzophenone moiety</td>
</tr>
<tr>
<td></td>
<td>640-800</td>
<td></td>
<td></td>
<td>Loss due to remaining organic moiety</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Zn residue</td>
</tr>
</tbody>
</table>
All the complexes indicated comparable pattern of decomposition mainly in three steps. Since the thermogram of all the complexes were found to be stable up to 200 °C indicating the absence of any crystalline/lattice water molecule which was further confirmed by the absence of any endothermic peak in the DTA curve in the range of 20-190 °C.\textsuperscript{61} In case of complex 1, the first decomposition step recorded in the range of 230-430 °C accompanied by weight loss calcld. 55.42% (Obs = 56.04%) which corresponds to loss of two chloride ion and benzophenone moiety supported by an endothermic DTA peak in the range of 250-400 °C. Further, decomposition starts in the range of 430-650 °C having weight loss calcld. 37.82% (Obs = 37.38%) supported by an exothermic DTA peak in the range of 480-620 °C due to decomposition of remaining part of organic moieties. However, beyond the 700 °C, horizontal thermal curve was observed due to formation of respective metal oxides.\textsuperscript{62}

4.4.7 SEM analysis of Schiff base compounds

The fundamental information of the properties such as microstructure, surface morphology and porosity were obtained from SEM micrographs.\textsuperscript{63} The comparisons of SEM micrographs of complexes are shown in Figure 4.7.

\textbf{Figure 4.7} SEM micrograph of Schiff base macrocyclic complexes of Co(II), (1); Ni(II), (2); Cu(II), (3); and Zn(II), (4); (a-d).
The change in surface morphology of the resultant complexes can be attributed to the coordination of the metal ions to the donor sites of ligand. The micrograph of complex 1 depicted porous honeycomb-like appearance while the micrographs of complex 2 displayed spherically agglomerated, dense structure. However, the micrograph of complex 3 depicted non-uniform flower-like clusters with interconnected network morphology whereas complex 4 was characterized by an aggregate of irregularly broken rocks-like shape.

4.4.8 Biological assessments

4.4.8.1 Steady state fluorescence

The fluorimetric competitive binding probes that can interact with nucleic acids play crucial role in biophysical studies of macromolecules with chemotherapeutic agents. Therefore, fluorescence quenching analysis using this probe affords additional information relating to the localization of the drugs and their mode of interaction with DNA. During fluorescent probe, EB (planar cationic dye) has been used since it does not emit fluorescence in view of the quenching effect of solvent molecules.

However, it has been already reported that EB in the presence of DNA emits intense fluorescence due to high degree of intercalation in between the adjacent base pairs of DNA.\textsuperscript{64} The partial quenching for this enhanced fluorescence can be observed by adding second molecule,\textsuperscript{65} which helps in binding mode determination. The evaluation of binding event of all the Schiff base macrocyclic complexes was performed by fluorescence-quenching experiments with EB-bound to DNA [Figure 4.8 (I), Table 4.2]. The quenching constant $K_{sv}$ was determined from the classical Stern-Volmer Eq. (4.3).\textsuperscript{66}

$$\frac{F_0}{F} = 1 + K_{sv} Q$$

where $F_0$ and $F$ are the fluorescence intensities in the absence and presence of complexes, respectively. The term $K_{sv}$ represents linear Stern-Volmer quenching constant while $[Q]$ is the concentration of the complexes (quencher).

The linear Stern-Volmer quenching constant $K_{sv}$ can be calculated from the slope to intercept ratio. The higher $K_{sv}$ value for the complex 3 ($7.75 \times 10^3$ M$^{-1}$) suggested its stronger ability to displace EB from DNA-EB system (Table 4.2).
Figure 4.8. (I) Fluorescence quenching curves of EB bound to CT-DNA by complexes 1-4 (a-d) at the excitation wavelength (478 nm). [DNA] = 50 μM, [EB] = 50 μM and [1-4] = 0-70 μM in DMSO-PBS buffer solution. The arrow shows the intensity change on increasing the complex concentration. Stern-Volmer plot (a’-d’) for interaction of complexes 1-4 with EB-DNA.
where \( k_q \) is the bimolecular rate constant of the quenching reaction and \( \tau_0 \) is the average lifespan of the biomolecule without a quencher and its value was \( \sim 10^{-8} \) s.\(^{67}\)

The \( k_q \) was observed in range of \( 2.09 \times 10^{11} - 7.75 \times 10^{11} \) (Table 4.2) which was greater than the limiting diffusion rate constant of the biomacromolecules \( (2.0 \times 10^{10} \text{ L mol}^{-1} \text{ s}^{-1}) \), implying that the static quenching process was observed rather than a dynamic one.\(^{68}\)

### 4.4.8.1.1 Calculation of Binding Constant

The decrease in the fluorescence intensity thus warrant the partial replacement of EB bound to DNA by complexes. Successive addition of the metal complexes (1-4) to EB-DNA system, remarkable decrease in emission intensity was recorded signifying the displacement of EB molecules by the metal complexes from their DNA binding sites. However, the comparative study revealed that complex 3 binds to DNA more strongly as compared to others.

For all the metal complexes with DNA in case of static quenching process, the binding constant \( (K_b) \) and binding sites number \( (n) \) is evaluated by the following Eq. (5).\(^{69}\)

\[
\log \left[ \frac{(F_0 - F)}{F_0} \right] = \log K_b + n \log[\text{DNA}] 
\]

The plots of \( \log \left[ \frac{(F_0 - F)}{F_0} \right] \) versus \( \log [Q] \) illustrated in Figure 4.8 (II) and the values of \( K_b \) and \( n \) are shown in Table 4.2. In addition, it is prominent that the binding constants ascertained for the typical classical intercalators (EB-DNA, \( 1.4 \times 10^6 \text{ M}^{-1} \)) are higher in comparison to the metal complexes\(^{70}\) (1-4).
Figure 4.8 (II) Plots of $\log[(F_0 - F)/F]$ versus $\log[Q]$ for the fluorescence quenching of DNA-EB system by complexes of Co(II), 1; Ni(II), 2; Cu(II), 3; and Zn(II), 4; (a-d).

Table 4.2 The Stern-Volmer constants ($K_{sv}$), linear correlation coefficient ($R^2$), limiting diffusion rate constant values ($k_q$), binding constant ($K_b$) and the number of binding sites per nucleotide ($n$) of complexes 1-4 with CT-DNA.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_{sv}$ (M$^{-1}$)</th>
<th>$R^2$</th>
<th>$k_q$ (M$^{-1}$s$^{-1}$)</th>
<th>$K_b$ (M$^{-1}$)</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex 1</td>
<td>$2.09 \times 10^3$</td>
<td>0.986</td>
<td>$2.09 \times 10^{11}$</td>
<td>$1.65 \times 10^3 \pm 0.057$</td>
<td>0.979</td>
</tr>
<tr>
<td>Complex 2</td>
<td>$2.99 \times 10^3$</td>
<td>0.946</td>
<td>$2.99 \times 10^{11}$</td>
<td>$3.19 \times 10^3 \pm 0.203$</td>
<td>1.016</td>
</tr>
<tr>
<td>Complex 3</td>
<td>$7.75 \times 10^3$</td>
<td>0.991</td>
<td>$7.75 \times 10^{11}$</td>
<td>$1.39 \times 10^4 \pm 0.020$</td>
<td>1.061</td>
</tr>
<tr>
<td>Complex 4</td>
<td>$4.32 \times 10^3$</td>
<td>0.991</td>
<td>$4.32 \times 10^{11}$</td>
<td>$2.71 \times 10^3 \pm 0.024$</td>
<td>0.954</td>
</tr>
</tbody>
</table>

4.4.8.2 Circular dichroic (CD) spectral studies

Circular dichroism is a technique used to monitor the interaction of nucleic acids with drugs, small molecules or metal complexes. CD spectrum of CT-DNA display a positive band and a negative band at 275 nm and 245 nm due to the base stacking and helicity, respectively which are the characteristic bands of B-form of CT-DNA recorded in the absence of the metal complexes. Factually, the molecules having the property of classical intercalation show a considerable shift in the intensities of both
the bands due to the base stacking interactions and stable conformations, while the electrostatic interaction and simple groove binding demonstrates less perturbation or no change in the base stacking helicity.\textsuperscript{73} To clarify the binding mode (intercalation or groove) of complexes (1-4) with CT-DNA, the CD spectrum was reported in the presence of varying concentration of complexes (Figure 4.9). The gradual addition of complex to the solution of CT-DNA does not cause any significant change in the intrinsic CD spectrum of the DNA indicating that binding of complexes with CT-DNA does not alter the stacking of bases. This observation rules out intercalative binding inferring the groove binding mode for all complexes with CT-DNA.

![Figure 4.9 CD spectra of CT-DNA (50 μM) in the absence and presence of complex of Co(II), 1 (A), Ni(II), 2 (B), Cu(II), 3 (C) and Zn (II), 4 (D) in DMSO-PBS buffer (pH = 7.20) at room temperature with varying concentration of compound 1-4 (0-20 μM).](image)

**4.4.8.3 DPPH free radical scavenging activity**

Since the newly synthesized schiff base macrocyclic complexes exhibited good DNA-binding affinity, they are expected to possess antioxidant property. Schiff base macrocyclic complexes were further screened for their *in-vitro* antioxidant activity for free-radical scavenging property by DPPH method at different concentration.\textsuperscript{74} The result varies directly with the different concentrations as shown in Figure 4.10.
Figure 4.10 Radical-scavenging activity on DPPH radicals (%) of the Schiff base synthesized macrocyclic compounds Co(II), 1; Ni(II), 2; Cu(II), 3 and Zn(II), 4.

All the analyses were done in duplicate and the results are averaged. The results exhibited good radical scavenger activity for all the metal complexes used in this study compared to the reference (Ascorbic acid). The highest inhibition percentage monitored in complex 3 was 64%, which was lower than that of reference at 450 µg/mL (80%). The activity difference of complexes may be accredited because of the coordination environment and redox properties. In general, a number of factors such as axial ligation, charge type, coordination number and chelate ring size, degree of unsaturation and chelate ring substitution pattern are responsible for the complex redox properties. Thus, it can be concluded that these metal complexes could be beneficial in improving their availability for therapeutic purposes.

The free-radical scavenging mechanism comprising reaction of $O_2^-$ reacted with Cu(II) complex, where only a small fraction of $O_2^-$ reacts with ligand while a large fraction reacts with Cu(II) and reduces Cu(II) to Cu(I). The subsequent oxidation results in the formation of parent Cu(II) complex with another $O_2^-$ molecule. This mechanism also explains the higher free-radical scavenging activity of Cu(II) complex compared with the other metal complexes.

In case of Zn(II) complex, it should be noted that imine group is present in ligand framework which exhibit non-innocent behaviour leading to the possibility of its participation in free radical scavenging property.
4.4.8.4 *In-vitro antimicrobial activity*

The metal complexes were comparatively screened against the bacterial strains as *E. coli*; gram -ve and *S. aureus*; gram +ve and fungal cells *C. albicans*. The zone of inhibition diameter (mm) was used for the comparative analysis of antimicrobial activity of the complexes (1-4) with the reference drug (*Figure 4.11 and Table 4.3*).

*Figure 4.11 Antibacterial (a) and antifungal activity (b) of Schiff base macrocyclic complexes of Co(II), (1); Ni(II), (2); Cu(II), (3) and Zn(II), (4).*
Table 4.3
(a) The inhibitory effects (in mm) and MIC (in μg/mL) of complexes (1-4) against bacterial strains using disk diffusion and micro broth dilution method, respectively.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Zone of inhibition (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. coli</em> (Gram-ve)</td>
</tr>
<tr>
<td>Complex 1</td>
<td>12.66 ± 0.57</td>
</tr>
<tr>
<td>Complex 2</td>
<td>11.66 ± 0.57</td>
</tr>
<tr>
<td>Complex 3</td>
<td>15.33 ± 1.52</td>
</tr>
<tr>
<td>Complex 4</td>
<td>9.33 ± 0.57</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>20.67 ± 1.67</td>
</tr>
</tbody>
</table>

**MIC (in μg/mL)**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>E. coli</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex 1</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>Complex 2</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>Complex 3</td>
<td>64</td>
<td>128</td>
</tr>
<tr>
<td>Complex 4</td>
<td>256</td>
<td>512</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>13</td>
<td>18</td>
</tr>
</tbody>
</table>

(b) The inhibitory effects (in mm) and MIC (in μg/mL) of complexes (1-4) against fungal strain using disk diffusion and the micro broth dilution method, respectively.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Zone of inhibition (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td>Complex 1</td>
<td>10.67 ± 0.57</td>
</tr>
<tr>
<td>Complex 2</td>
<td>9.3 ± 0.57</td>
</tr>
<tr>
<td>Complex 3</td>
<td>8.33 ± 1.15</td>
</tr>
<tr>
<td>Complex 4</td>
<td>8.67 ± 1.1</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>18 ± 0.33</td>
</tr>
</tbody>
</table>

**MIC (in μg/mL)**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>E. coli</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex 1</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Complex 2</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Complex 3</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>Complex 4</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>12.50</td>
<td></td>
</tr>
</tbody>
</table>
Furthermore, in order to compare the MIC’s of the complexes (1-4) against different strains in comparison to reference drug are presented in Table 4.3 which represent that the complexes possess higher zone of inhibition showing lower MIC’s and hence act as active antibacterial and antifungal agents. The better antibacterial activity was shown by complex 3 which proved their usefulness as potential broad-spectrum antimicrobial agents.

The antibacterial activity of the complexes in terms of structural changes and reactivity, induced by in-situ coordination of ligand moiety with the metal ion may be understood in terms of chelation theory. As a matter of fact chelation/coordination reduces the polarity of the metal ion because of the partial sharing of its positive charge with donor group within the whole chelate ring system. Hence, chelation process enhances the lipophilic nature of the central metal atom, favoring its permeation through the lipid layer of the membrane causing metal complex to cross the bacterial membrane more effectively, blocking the metal binding sites of microorganisms. This inhibits the synthesis of the proteins by the complexes which actually restrict the further growth of the microorganisms raising its antibacterial activity.

4.4.8.5 Effect of schiff base compound on bacteria
The Schiff base macrocyclic compound 3, disrupt the cell morphology in E. coli, S. aureus and C. albicans. The SEM images of treated cells clearly indicated the interference of complex 3 with cell membrane integrity, as evidenced by shrinkage of the cell surface in biofilm cells without changing the architectural integrity of biofilm and morphology of the cells in control (Figure 4.12).
Figure 4.12 SEM images showing the effect of Schiff base macrocyclic complex of Cu(II); 3 on bacterial cell (a) Normal E. coli cells (a’)
Effect after complex 3 (b) Normal S. aureus cells (b’)
Effect after complex 3 (c) Normal C. albicans cells (c’)
Effect after complex 3.
4.4.8.6 **Biofilm inhibition**

The biofilms acts as double-edged sword being resistant towards antibiotics and immunological barriers. The XTT assay was done quantitatively to assess the potential of complex 3 against developing biofilms of *E. coli*, *S. aureus* and *C. albicans* \(^1\) (**Figure 4.13**).

![Figure 4.13 Determination of % biofilm inhibition of synthesized Schiff base macrocyclic complex of Cu(II), 3 on E. coli (a), S. aureus (b) and C. albicans (c) by XTT method.](image)

This study revealed a varying level of depletion of biofilm formation by respective bacterial and yeast cells in the presence of complex 3 in a dose-dependent manner. The complex 3 at the concentration of 200 µg/mL exhibited 77% and 71% inhibition on biofilm formation of *E. coli* and *S. aureus*, respectively, while the same complex at a concentration of 500 µg/mL showed 71% inhibition. Furthermore, the comparative study suggested that complex 3 showed the more inhibition against *S. aureus* biofilm.
### 4.4.8.7 Docking Study

Molecular docking is an attractive computational practice to study the interactions between the drug and DNA for rational drug designing application also for the mechanistic analysis by placing a small molecule inside the binding site of the target specific region of the DNA usually in a non-covalent mode which can validate the spectroscopic results. Molecular modeling provides the flexibility within the ligand to be modeled and can utilize more detailed knowledge about the molecular mechanics to numerate the energy of the ligand in context of the putative active site. To corroborate the groove binding mode, structure of the metal complexes (3 and 4) was made flexible to acquire different conformations to predict the preferred binding site of 3 and 4 with DNA duplex of sequence d(CGCGAATTCGCG)₂ (PDB ID: 1BNA) which affords an energetically favorable docked pose (Figure 4.14). Herein, the comparative resulting docked model displayed both the complexes fit adequately into the curved contour of the targeted DNA in the minor groove through aromatic rings located within narrower G-C rich region as compared to A-T regions leading to number of non bonding interaction such as van der Waals interaction and hydrophobic contacts with DNA functional groups that define the stability of groove. Eminently, the interactions of chemical species into the minor groove of B-DNA vary from those securing in the major groove, both in terms of the electrostatic potential and steric effects in view of the narrow shape of the former. In comparison to the major groove, due to small steric interference small molecules preferentially interact within the minor groove. The minimum binding energies of the docked metal complexes (3 and 4) were found to be -4.0 and -3.6 kcal/mol, respectively. The more negative relative binding energy of complex 3 indicates more potent binding between DNA and target molecules. Thus, the experimental results obtained from spectroscopic techniques substantiate the results scored from the molecular docking, hence providing further evidence of groove binding.
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Figure 4.14 Molecular docked structures of Schiff base macrocyclic compound 3, Cu(II) (a, b, c) and 4, Zn(II) (d, e, f) complexed with DNA dodecamer duplex of sequence d(CGCGAATTCGCG)₂ (PDB ID:1BNA). Stereoview (A, B) of the docked conformation of complex-DNA (3 and 4) showing the possibility of hydrogen bonds.

4.5 CONCLUSION

12 membered Schiff base macrocyclic metal complexes were prepared with isatin and 3,4-diaminobenzophenone and characterized by various spectroscopic and physicochemical techniques which confirmed the formation, stoichiometry and bonding nature. Further, the comparative DNA-binding studies revealed the greater complex-DNA interaction for Cu(II) complex; 3 with the exploration of the groove binding mode approved by circular dichroism and docking studies. In addition, the DPPH radical scavenging activity results proved that these complexes exhibited scavenging potential. The synthesized complexes possess significant potential of antibacterial activity against different bacterial and fungal strains. Hence, in account of these observations, it is concluded that the promising binding and biological nature of the Cu(II) complex could pave way for designing more effective metal-based drugs.
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4.6 REFERENCES


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