

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF BROMO-SUBSTITUTED SCHIFF BASE METAL COMPLEXES DERIVED FROM SALICYLALDEHYDE

O.J. Manjubhargavi^{1*}, S. Sree Brindha², S. Karthikeyan³, R. Prabhakaran⁴

¹Department of Chemistry, Padhmashree Institute of Management and Sciences, Bangalore, India, manjubhargavi.oj@gmail.com

²Department of Chemistry, Bharathiar University, Coimbatore, 641 046, India.

³Department of Chemistry, Chikkaiah Government Arts & Sciences College, Erode, 638 004, India.

⁴Department of Chemistry, Padhmashree Institute of Management and Sciences, Bangalore, India

ABSTRACT:

Bromo-substituted Schiff base ligand derived from salicylaldehyde and 4-bromoaniline was synthesized and complexed with Fe(II), Cu(II), Zn(II), Cd(II), and Hg(II) ions. The ligand and its metal complexes were characterized using UV-Vis, FT-IR, and NMR spectroscopic techniques, confirming coordination through azomethine nitrogen and phenolic oxygen atoms. Antioxidant potential was evaluated by nitric oxide and DPPH radical scavenging assays, revealing moderate to appreciable activity for selected complexes. In silico target prediction indicated a strong affinity toward carbonic anhydrase isoforms and other cancer-relevant targets. The study highlights the role of halogen substitution and metal coordination in modulating biological activity of Schiff base complexes.

KEYWORDS: Schiff base ligand, Salicylaldehyde, Bromo-substituted aromatic amine, Transition metal complexes, Spectroscopic characterization, Antioxidant activity, DPPH radical scavenging, Nitric oxide scavenging, Halogen substitution effect, In silico target prediction.

INTRODUCTION

Schiff base ligands derived from aromatic amines and salicylaldehyde continue to occupy a prominent position in coordination and bioinorganic chemistry owing to their strong metal-chelating ability, structural rigidity, and pronounced biological relevance. The azomethine ($-\text{CH}=\text{N}-$) linkage, in conjunction with the phenolic oxygen atom of salicylaldehyde, provides an efficient N,O-donor system capable of stabilizing a wide range of metal ions. Such ligands readily form stable chelates with transition and post-transition metals, often resulting in enhanced physicochemical properties and improved biological activity compared to their uncoordinated counterparts. Schiff base metal complexes have been widely reported for their antioxidant and biomolecular interaction properties, making them promising candidates in therapeutic and pharmaceutical research.

Drawing from the outcomes of the earlier chapters, which demonstrated how ligand architecture and substituent effects significantly influence coordination behavior and biological performance, the present chapter extends the investigation to a halogen-substituted aromatic Schiff base system. This progression is intended to provide a comparative understanding of how heterocyclic substitution and halogen substitution differentially affect metal-ligand interactions and bioactivity. Accordingly, the current study employs 4-bromoaniline as the amine component and salicylaldehyde as the aldehyde precursor to synthesize a bromo-substituted Schiff base ligand.

The presence of a bromine substituent on the aromatic ring is of particular interest due to its electron-withdrawing nature and its known influence on lipophilicity, molecular stability, and biological interactions. Halogen substitution has frequently been associated with enhanced antioxidant activity and improved protein-binding affinity, owing to increased hydrophobic interactions and favorable electronic effects. The synthesized ligand possesses the characteristic azomethine nitrogen and phenolic oxygen donor atoms, enabling it to act as an efficient chelating agent toward metal ions.

Subsequently, the ligand was complexed with Fe(II), Cu(II), Zn(II), Cd(II), and Hg(II) ions under controlled reaction conditions. The selection of Fe(II) is particularly significant due to its central role in redox processes and biological electron-transfer systems, while Cu(II) and Zn(II) are well known for their involvement in enzymatic and antioxidant mechanisms. Cd(II) and Hg(II) were included for comparative purposes to examine the influence of heavier metal ions on coordination strength and biomolecular interaction. Metal complex formation is expected to modify electronic distribution and redox behavior, thereby influencing biological activity in accordance with chelation theory.

The biological investigations in this chapter focus on antioxidant activity evaluated through nitric oxide scavenging and DPPH free radical scavenging assays, along with protein-binding studies. Nitric oxide scavenging activity provides insight into the potential of the compounds to modulate reactive nitrogen species implicated in oxidative stress and inflammatory conditions. The DPPH assay serves as a reliable measure of free radical neutralization efficiency, while protein-binding studies offer valuable information on the interaction of the complexes with biological macromolecules, an essential factor influencing bioavailability and pharmacological behavior.

This chapter aims to elucidate the influence of halogen substitution and selective metal ion coordination on the antioxidant and biomolecular interaction properties of salicylaldehyde-derived Schiff base complexes. The findings are

expected to complement the previous chapters and contribute to a comprehensive understanding of structure–activity relationships in Schiff base metal complexes, reinforcing their relevance in bioinorganic and medicinal chemistry.

MATERIALS AND METHODS

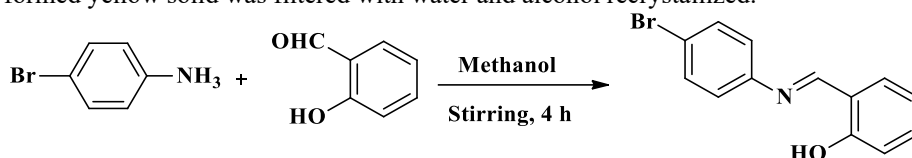
All the chemicals used were of purely analytical grade. Solvents were purified and dried according to standard procedure. MCF-7 Cell lines were obtained from the Protein Design private limited company, Mathikere, Bangalore, The cells were maintained in Dulbecco's modified eagle medium (DMEM, Sigma), 10% fetal bovine serum (Thermo), penicillin/streptomycin, Glutamine, incubated for 48 hours for growth in a humidified atmosphere of 95% air and 5% CO₂ to 80 percent confluency in a 25 mm flask.

Physical measurements

The Electronic spectra were measured by using JASCO 600 spectrophotometer in DMSO solution in the 200-800 nm range. The FT-IR spectra were recorded on a Thermo Nicolet Avatar FT-IR spectrometer as Potassium Bromide powder in the frequency range 400–4000 cm⁻¹. ¹H NMR and ¹³C NMR spectra were recorded in Bruker AV 400 instrument.

Synthesis of Schiff base Ligand

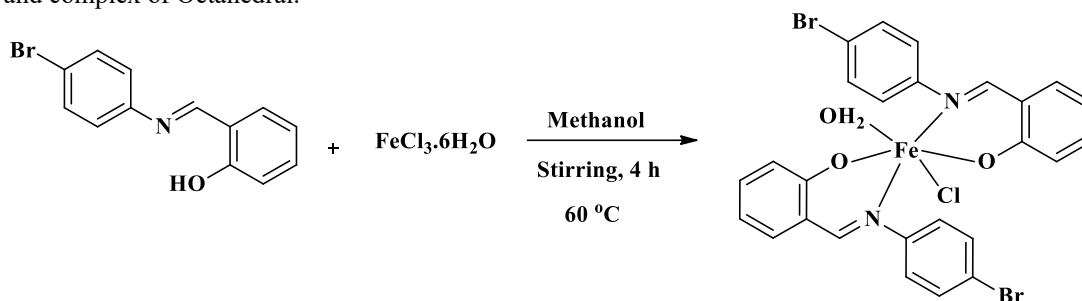
The reaction of equimolar ratios of Salicylaldehyde and 4-bromoaniline in good development has synthesized the ligand. The ligand was synthesized, is pale yellow in color (Munde et al.). In typical solvents which is organic such as CH₃OH, CH₃CN, C₆H₆, DMSO, DMF and CHCl₃, the ligand was soluble. In a methanol solution of 4-chloro aniline was added a solution of salicylaldehyde (10 cm³). At a temperature of 60°C, the mixture was stirred for 4 hours. The formed yellow solid was filtered with water and alcohol recrystallized.



Synthesis of Schiff base metal complexes

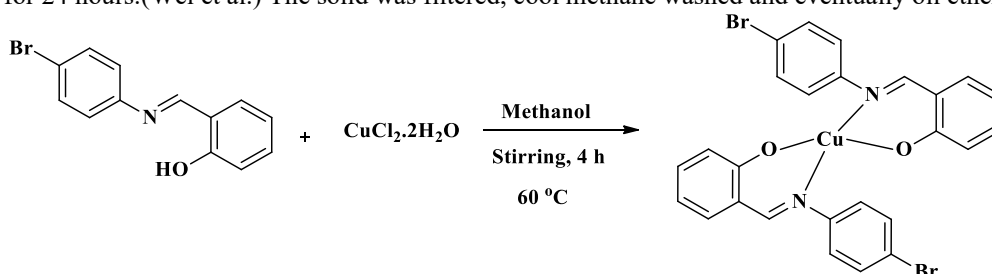
Synthesis of Iron Schiff base complex

FeCl₃.6H₂O solution is taken in a minimal volume of H₂O and applied to a solution of ligand in 30 cm³ of methanol. In reflux conditions the mixture was agitated for 4 hours. The mixture has been preserved in 24 hours contact with the mother oil. (Basak et al.) The solid was distilled, cold methanol washed and eventually oil ether washed. M.P. 400 °C and complex of Octahedral.



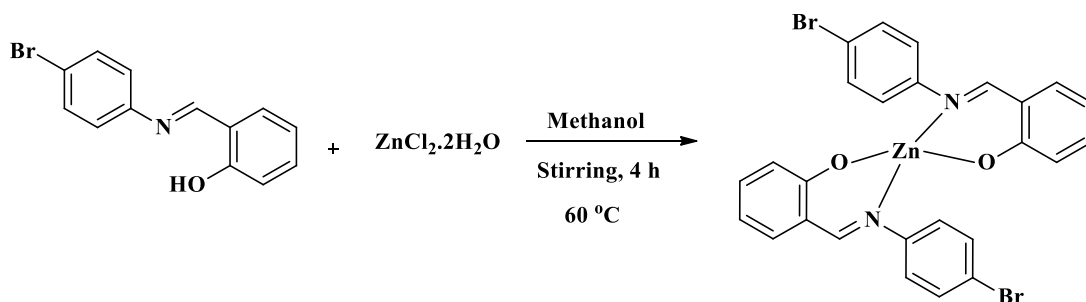
Copper Schiff base complex Synthesis

A solution of CuCl₂.2H₂O in a for the solution of ligand in 30 cm³ of methanol the appropriate volume of water was added. In reflux mode, the mixture was removed for 4 hours. The blend has been kept in contact with the liquid mother for 24 hours. (Wei et al.) The solid was filtered, cool methane washed and eventually oil ether washed in the dark brown.



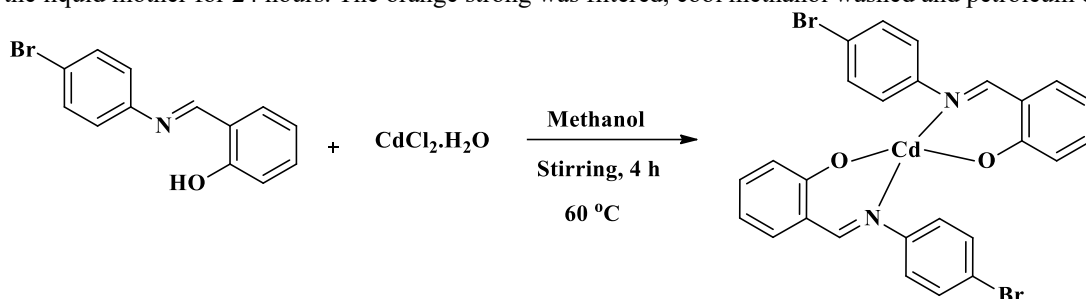
Synthesis of Zinc Schiff base complex

A ZnCl₂ solution in minimal water quantity was applied to a solution of ligand in 30 cm³ methanol. (Yu et al.) In reflux mode, the mixture was removed for 4 hours. The blend has been kept in contact with the liquid mother for 24 hours. The light-yellow solid has been filtered, cold methanol is cleaned and eventually oil ether has been added.



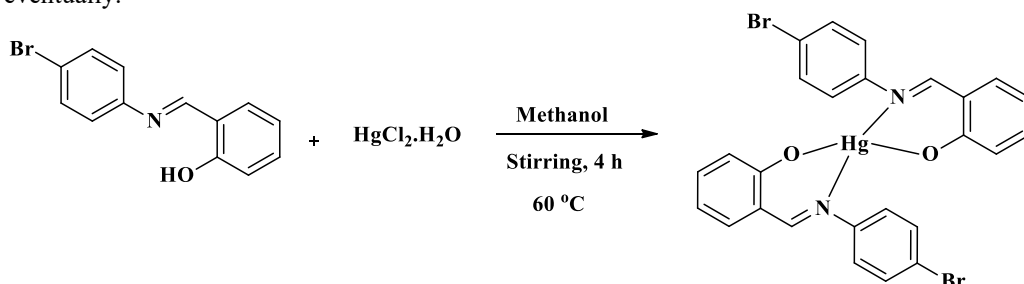
Synthesis of Cadmium Schiff base complex

A CdCl₂ solution with a minimal quantity of water was applied falling carefully to a ligand solution in 30 cm³ methanol. (Golcu et al.) In reflux mode, the mixture was removed for 4 hours. The blend has been kept in contact with the liquid mother for 24 hours. The orange strong was filtered, cool methanol washed and petroleum ether eventually.



Synthesis of Mercury Schiff base complex

A HgCl₂ solution with a minimal volume of water was applied with a decrease wise to a ligand solution in 30 cm³ methanol. (Al Zoubi et al.) In reflux conditions the mixture was agitated for 4 hours. The blend has been kept in contact with the liquid mother for 24 hours. The orange strong was filtered, cool methanol washed and petroleum ether eventually.

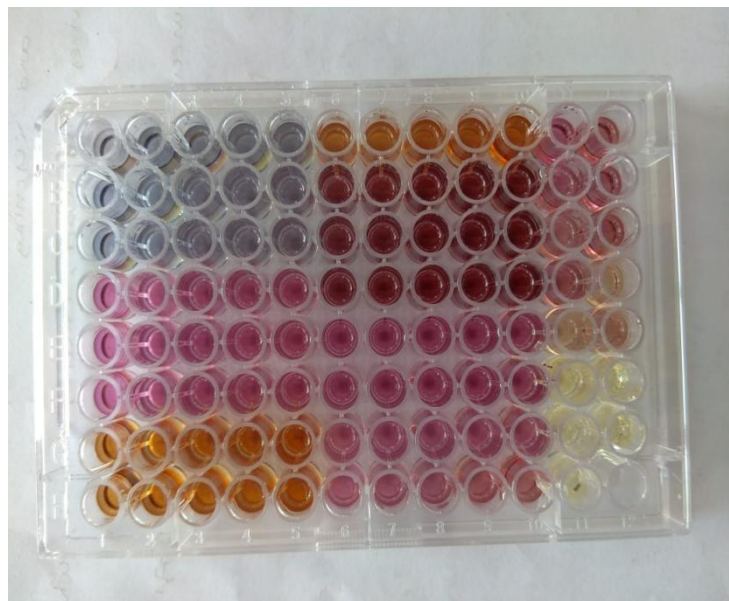


Procedure for antioxidant studies

Nitric oxide radical scavenging activity

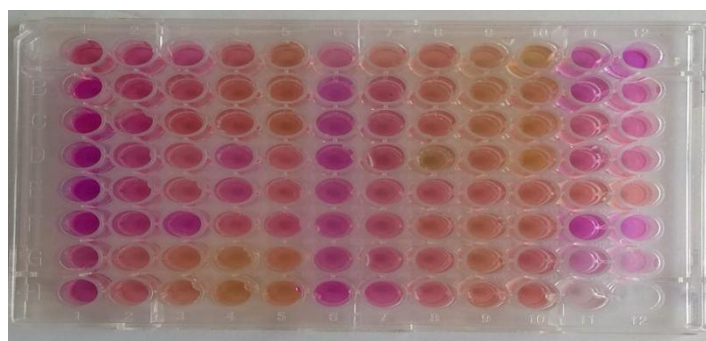
Nitric oxide (NO) radical is generated from sodium nitroprusside solution at physiological Ph. Sodium nitroprusside (1ml of 10 Mm) is mixed with 1ml of test compounds at different concentration (10-50 µg/ml) in phosphate buffer (Ph 7.4). The mixture is incubated at 25°C for 150 min. To 1 ml of the incubated solution, 1ml of Griess's reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) is added. Absorbance is read at 546 nm. Ascorbic acid was used as standard drug material.





Procedure for DPPH Free radical scavenging activity

The free radical scavenging activity of the title compounds was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. 0.2 Mm solution of DPPH in methanol was prepared and 100 μ l of this solution was added to various concentrations of title compounds at the concentrations of 10, 20, 30, 40, 50 μ g/ml. After 30 minutes, absorbance was measured at 517nm. Ascorbic acid was used as the reference material.



Procedure for In silico Target Prediction Procedure

The potential biological targets of the synthesized compounds were predicted using a ligand-based computational target prediction approach. The chemical structures of the compounds were first drawn using ChemDraw software and subsequently converted into three-dimensional formats. The optimized structures were saved in Structure Data File (SDF) or SMILES format and used as input for target prediction analysis. The prediction was performed using the SwissTargetPrediction platform, which identifies probable protein targets based on combined two-dimensional and three-dimensional structural similarity comparisons with known ligands deposited in the ChEMBL database.

The analysis was restricted to the human target set to ensure biological relevance. For each compound, predicted targets were ranked according to probability scores generated by the algorithm, which reflect the likelihood of interaction between the compound and a given target. Target information including common name, UniProt ID, ChEMBL ID, target class, prediction probability, and the number of known active ligands based on three-dimensional and two-dimensional similarity was retrieved. The targets with the highest probability values were selected and compiled to generate the final target prediction table for interpretation.

RESULTS AND DISCUSSION

UV-vis spectra

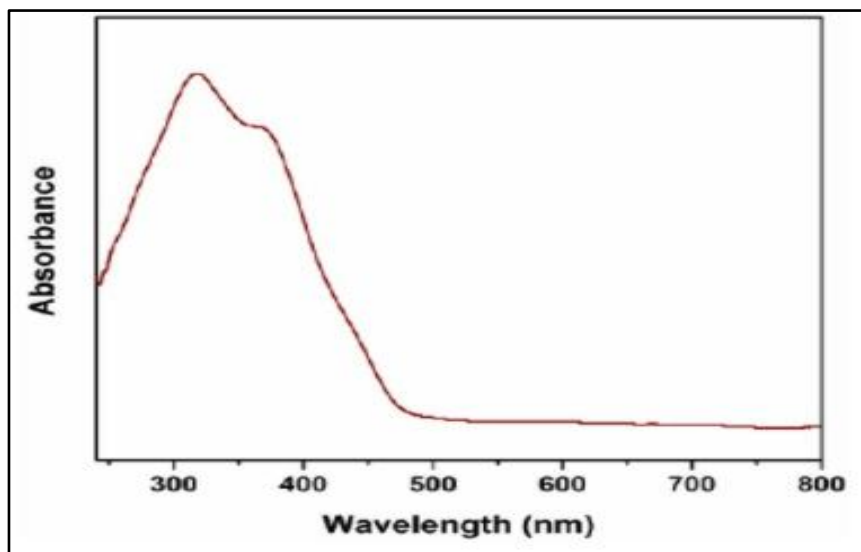


Figure 1 UV Spectrum of Ligand

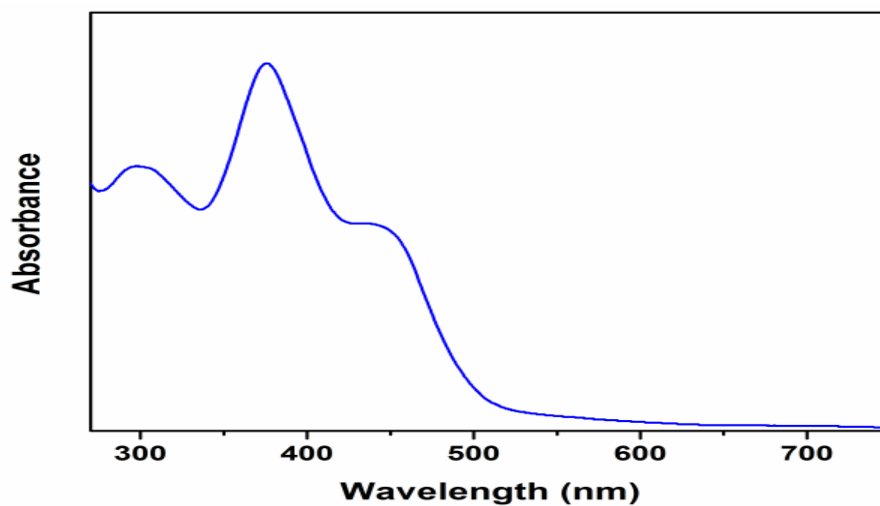


Figure 2. UV Spectrum of complex Iron (Fe)

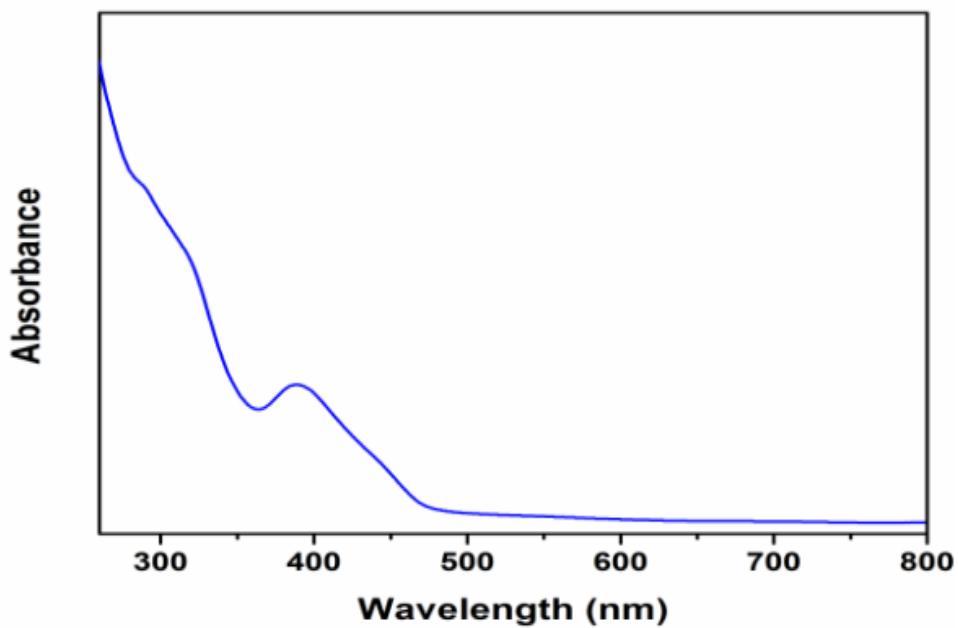


Figure 3. UV Spectrum of Cu complex

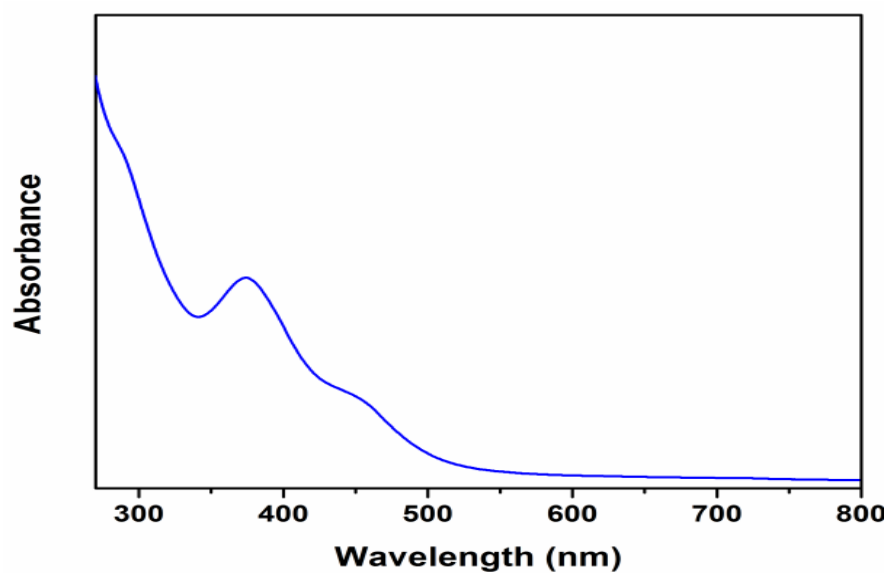


Figure 4. UV Spectrum of Zn complex

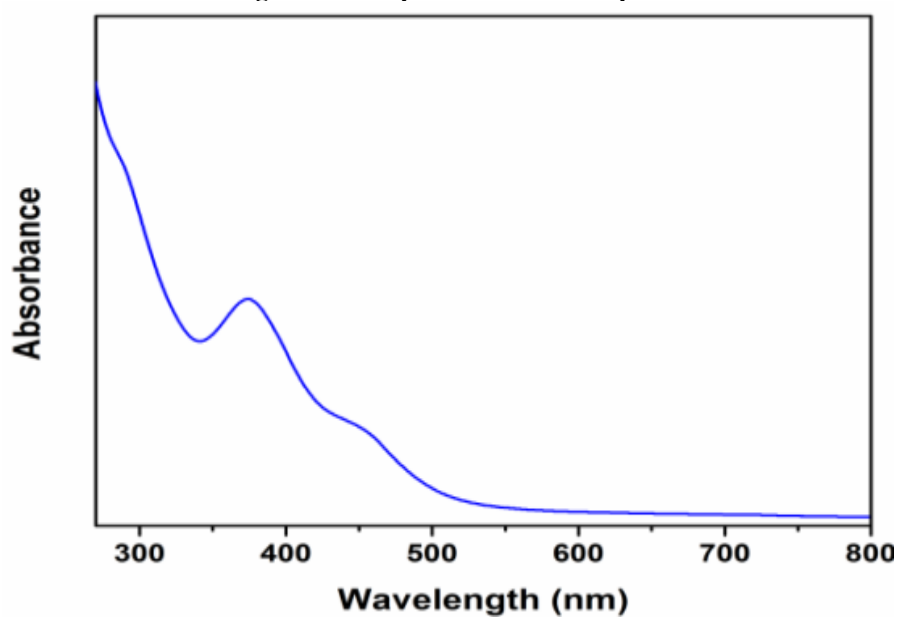


Figure 5. UV Spectrum Cd complex

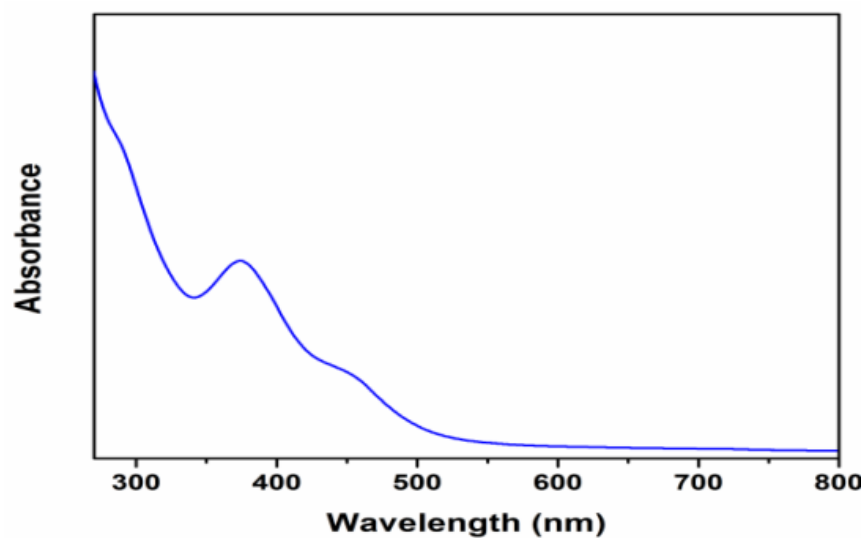


Figure 6. . UV Spectrum of Hg complex

IR spectra

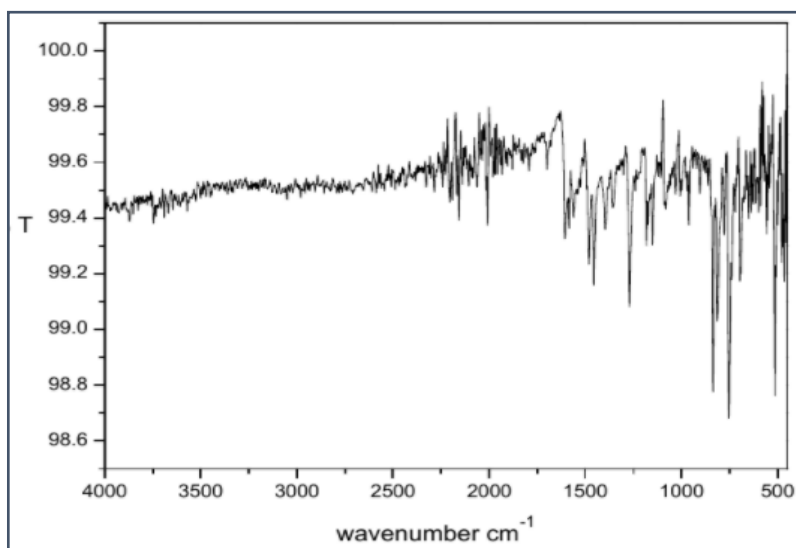


Figure 7. IR Spectrum of Ligand

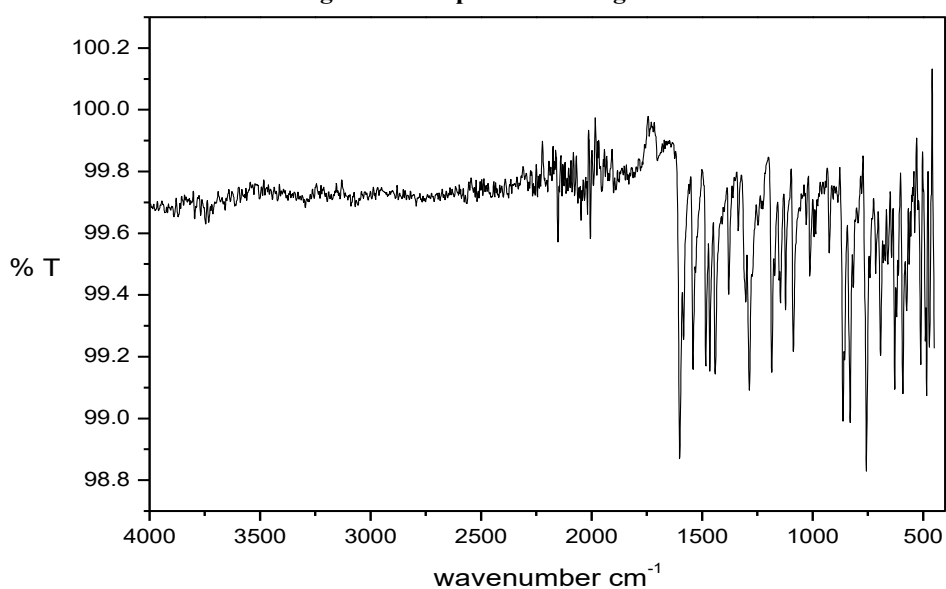


Figure 8. IR Spectrum of Fe complex

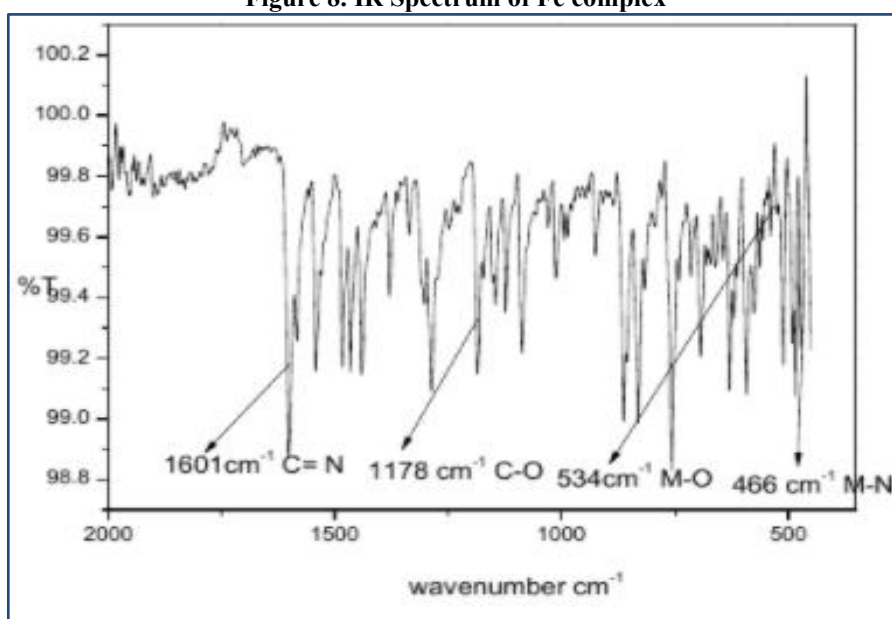


Figure 9. Fe complex expanded IR Spectrum

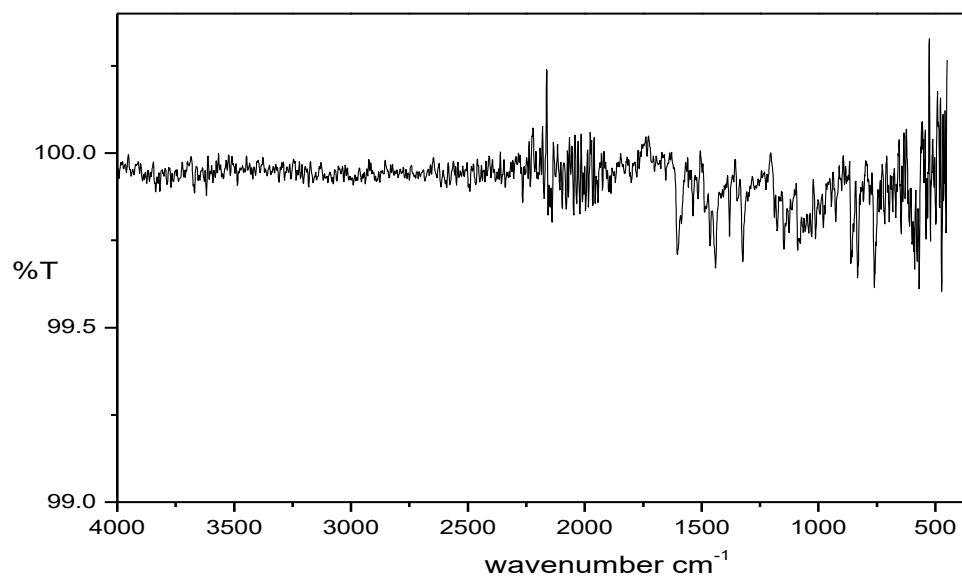


Figure 10. Cu complex IR Spectrum

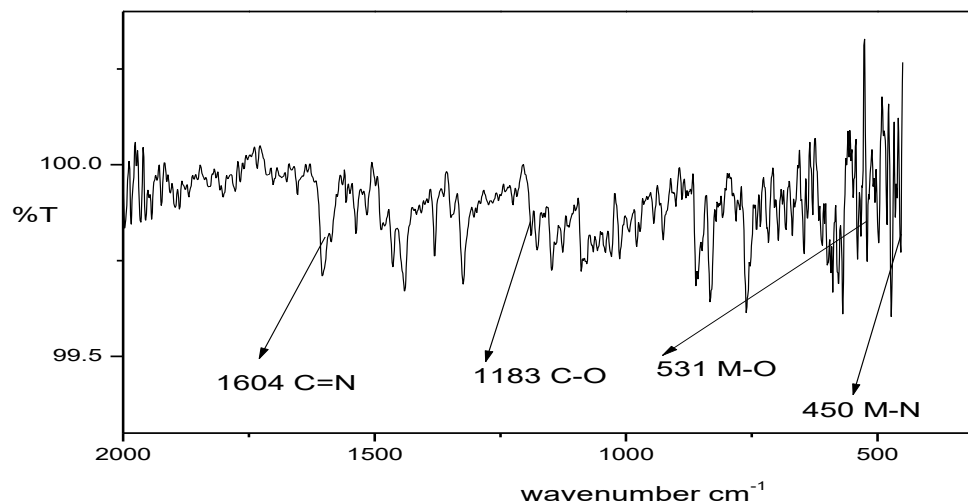


Figure 11. Cu complex expanded IR SPECTRUM

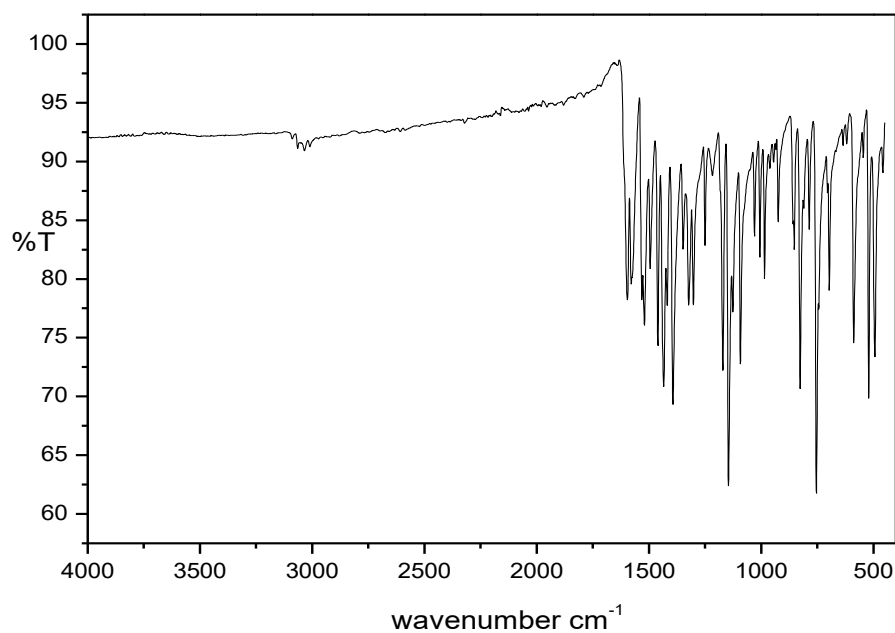


Figure 12. Zn complex IR SPECTRUM

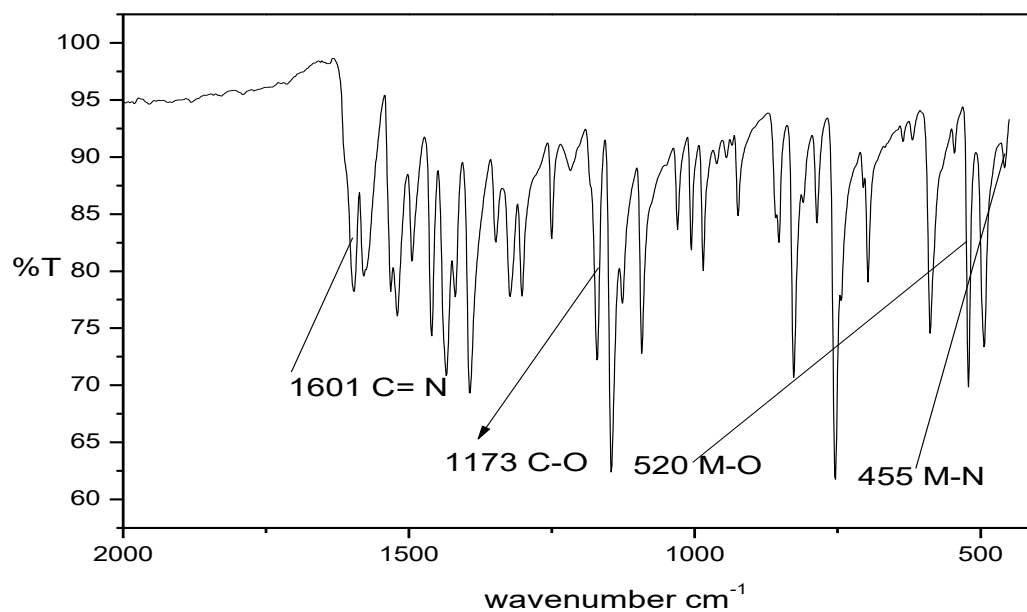


Figure 13. Zn complex expanded IR SPECTRUM

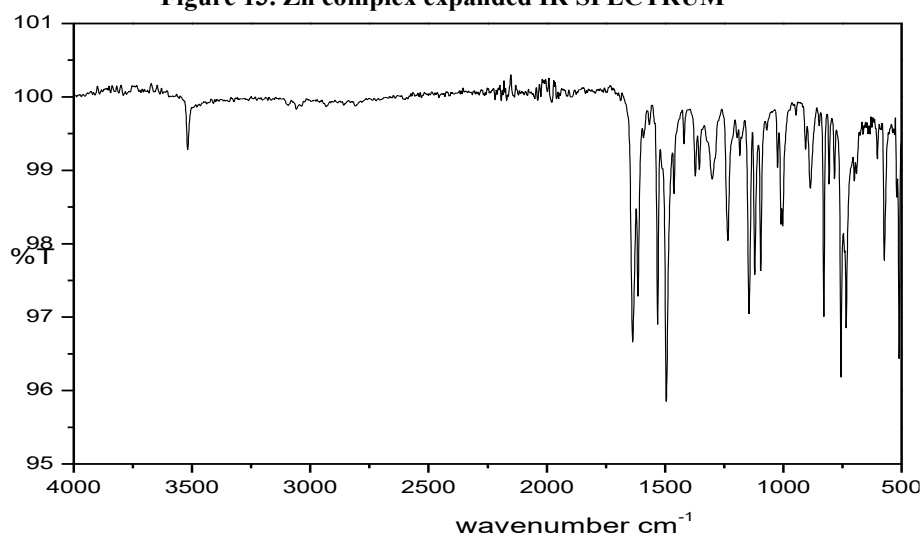


Figure 14. Cd complex IR SPECTRUM

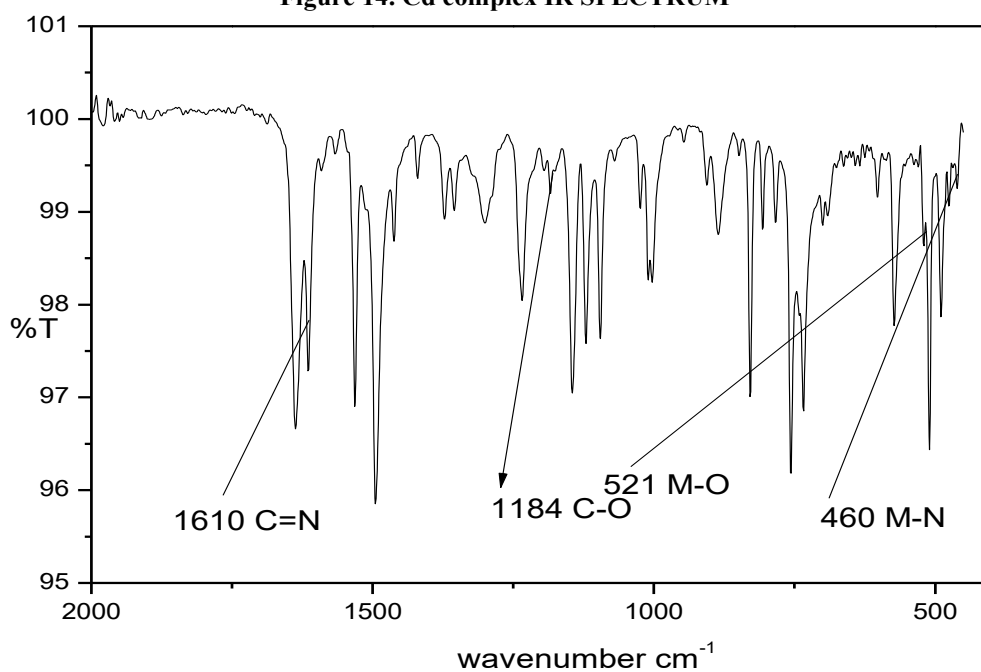


Figure 15. Cd complex expanded IR SPECTRUM EXPANDED

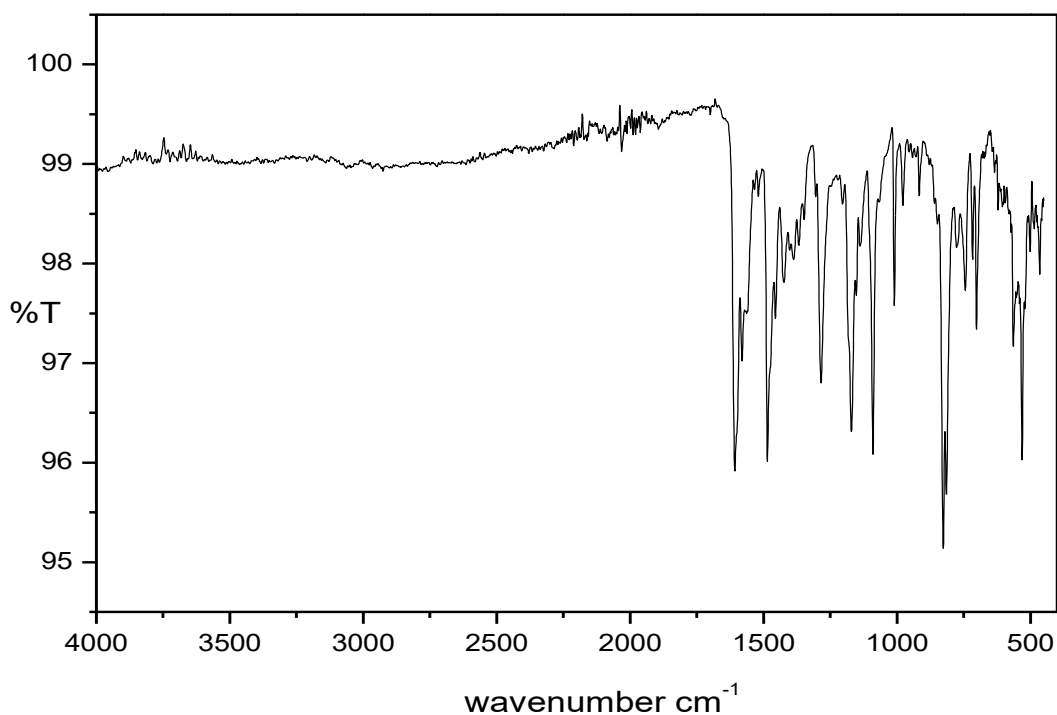


Figure 16. Hg complex IR SPECTRUM

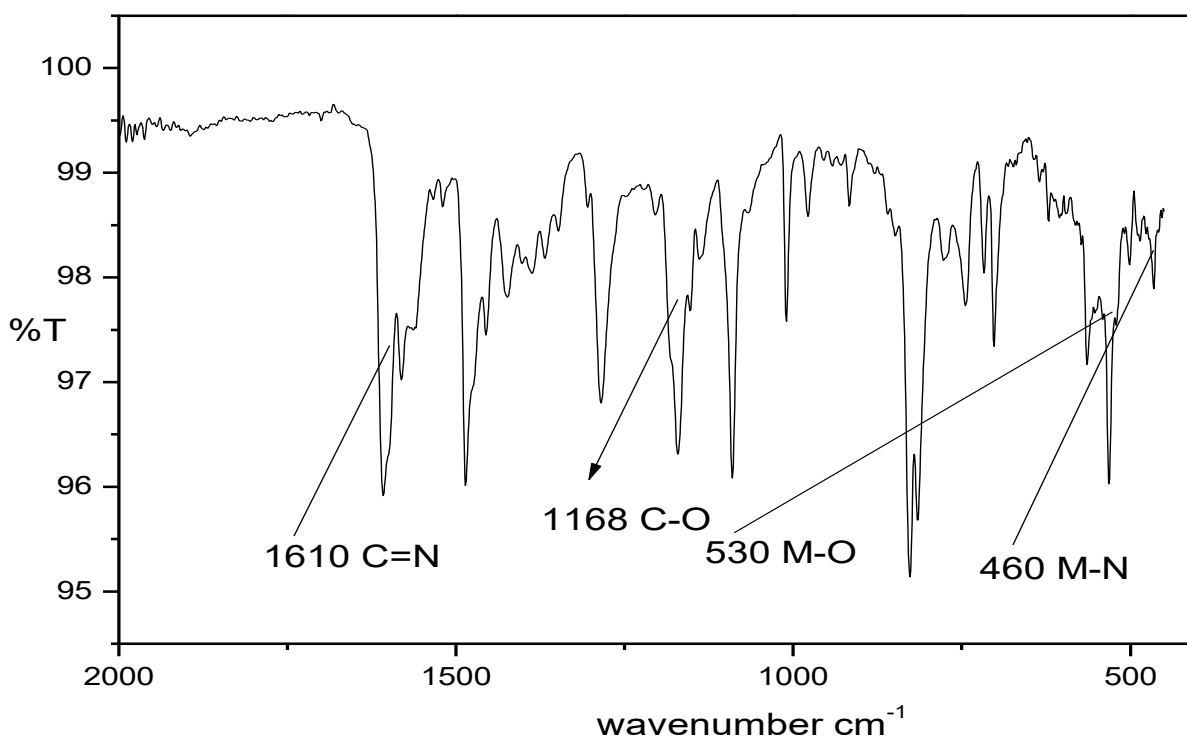
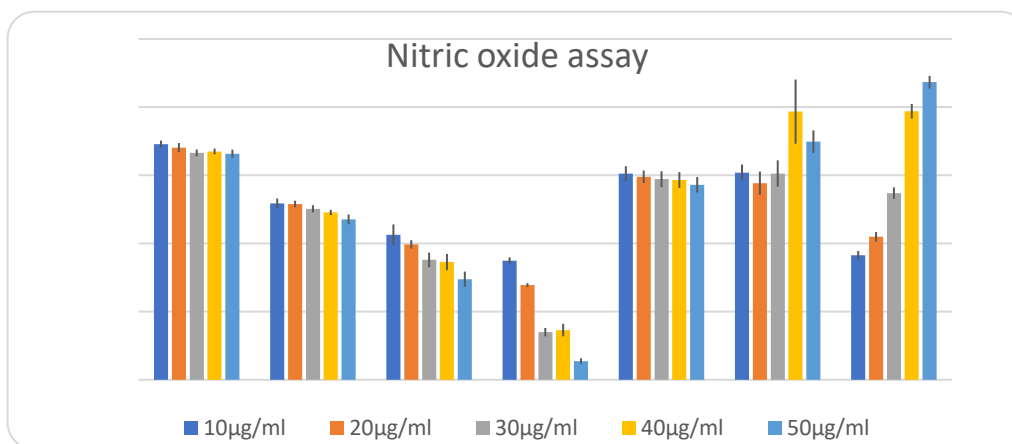


Figure 17. Hg complex expanded IR Spectrum

5.3.5 Result for Nitric oxide antioxidant activity

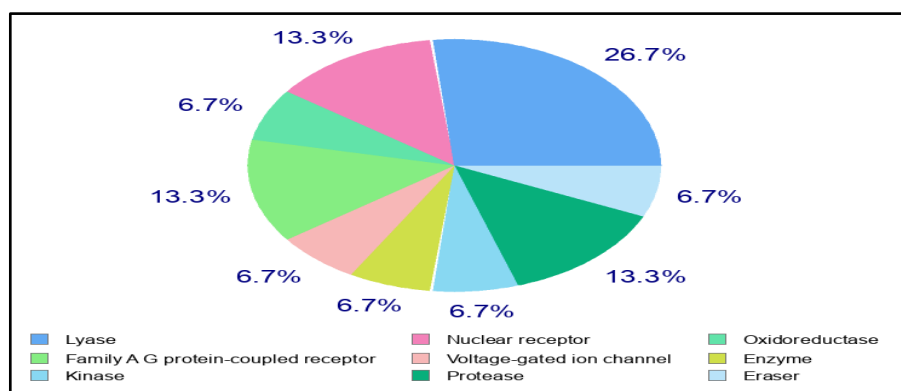
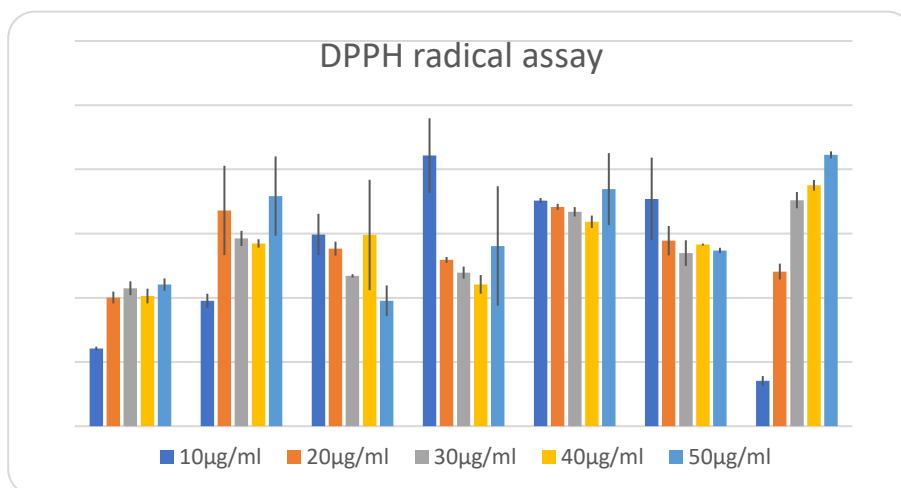
Comp	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	IC50
Cu	69.16±1.05	68.15±1.32	66.57±1.02	67±0.85	66.34±1.22	18.3
Cd	51.75±1.46	51.59±0.96	50.17±1.06	49.1±0.79	47.09±1.42	37.98
Co	42.59±3.01	39.75±1.22	35.19±2.15	34.53±2.39	29.52±2.23	83.12
Fe	34.97±0.98	27.8±0.57	13.99±1.22	14.6±1.85	5.5±0.83	24.89
Hg	60.49±2.19	59.55±1.85	58.87±2.34	58.64±2.32	57.24±2.28	26.09

Zn	60.82±2.36	57.7±3.43	60.54±3.85	78.7±9.43	69.9±3.29	21.03
STD (Ascorbic acid)	36.54±1.25	41.96±1.41	54.77±1.69	78.8±2.15	87.34±1.84	25.06



5.3.6 Results of DPPH Radical Scavenging Activity

Comp	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	IC50
Cu	24.19±0.6	40.08±1.84	42.96±2.15	40.56±2.29	44.11±1.94	48.14
Cd	39.06±2.19	67.22±13.91	58.51±2.36	56.93±1.31	71.68±12.36	25.96
Co	59.73±6.43	55.31±2.18	46.83±0.52	59.57±17.21	39.1±4.75	37.34
Fe	84.3±11.62	51.8±0.93	47.86±1.87	44.19±2.91	56.14±18.59	30.86
Hg	70.3±0.78	68.33±0.95	66.79±1.47	63.67±1.94	73.85±11.22	17.8
Zn	70.81±12.85	57.8±4.59	53.93±3.99	56.57±0.3	54.76±0.83	26.58
STD (Ascorbic acid)	14.09±1.55	48.17±2.47	70.42±2.54	75.03±1.68	84.5±1.11	25.73



Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability*	Known actives (3D/2D)
Carbonic anhydrase II	CA2	P00918	CHEMBL205	Lyase	0.086443	415 / 24
Carbonic anhydrase XII	CA12	O43570	CHEMBL3242	Lyase	0.086443	240 / 14
Carbonic anhydrase IX	CA9	Q16790	CHEMBL3594	Lyase	0.086443	292 / 12
Estrogen receptor beta	ESR2	Q92731	CHEMBL242	Nuclear receptor	0.077758	34 / 25
Dihydroorotate dehydrogenase	DHODH	Q02127	CHEMBL1966	Oxidoreductase	0.069097	9 / 2
Estrogen receptor alpha	ESR1	P03372	CHEMBL206	Nuclear receptor	0.069097	21 / 21
Adenosine receptor A1	ADORA1	P30542	CHEMBL226	Family A G protein-coupled receptor	0.060425	369 / 0
Adenosine receptor A2a	ADORA2A	P29274	CHEMBL251	Family A G protein-coupled receptor	0.060425	260 / 0
Calcium-activated potassium channel subunit alpha-1	KCNMA1	Q12791	CHEMBL4304	Voltage-gated ion channel	0.060425	13 / 0
Estradiol 17-beta-dehydrogenase 2	HSD17B2	P37059	CHEMBL2789	Enzyme	0.060425	21 / 0
Vascular endothelial growth factor receptor 2	KDR	P35968	CHEMBL279	Kinase	0.060425	397 / 0
Carbonic anhydrase I	CA1	P00915	CHEMBL261	Lyase	0.060425	343 / 16
Cathepsin (V and K)	CTSV	O60911	CHEMBL3272	Protease	0.060425	18 / 0
Cathepsin L	CTSL	P07711	CHEMBL3837	Protease	0.060425	121 / 0
NAD-dependent deacetylase sirtuin 1	SIRT1	Q96EB6	CHEMBL4506	Eraser	0.060425	9 / 0
Fatty acid desaturase 1	FADS1	O60427	CHEMBL5840	Enzyme	0.060425	8 / 0
Matrix metalloproteinase 9	MMP9	P14780	CHEMBL321	Protease	0.060425	57 / 0
Matrix metalloproteinase 2	MMP2	P08253	CHEMBL333	Protease	0.060425	60 / 0
L-lactate dehydrogenase chain	LDHA	P00338	CHEMBL4835	Enzyme	0.060425	8 / 0
Arachidonate 5-lipoxygenase	ALOX5	P09917	CHEMBL215	Oxidoreductase	0.060425	85 / 0

The above table summarizes the predicted biological targets of the studied compound(s), along with their corresponding target classes, prediction probabilities, and the number of known active ligands. The highest prediction probabilities (0.086443) were observed for carbonic anhydrase isoforms CA2, CA12, and CA9, indicating that these lyase enzymes are the most likely molecular targets. Among them, CA2 shows the largest number of known actives, particularly in three-dimensional data sets, suggesting strong prior experimental support and relevance for ligand binding. This highlights a potential preference of the compound(s) toward carbonic anhydrase inhibition, which is commonly associated with anticancer and antiangiogenic activities.

Moderate probabilities were predicted for estrogen receptors ESR2 and ESR1, belonging to the nuclear receptor class, suggesting possible hormone receptor modulation. Additional targets with slightly lower but comparable probabilities include DHODH, adenosine receptors (ADORA1 and ADORA2A), VEGFR-2 (KDR), cathepsins, matrix metalloproteinases (MMP2 and MMP9), and metabolic enzymes such as LDHA and ALOX5. These targets are involved

in key biological processes including cell proliferation, angiogenesis, inflammation, metabolism, and extracellular matrix remodeling. Overall, the results indicate a multi-target interaction profile, with a pronounced tendency toward carbonic anhydrase isoforms, supporting the potential therapeutic relevance of the compounds in cancer-related and metabolic pathways.

ChE MBL ID	Name	UniProt Accession	Type	Organis m	Compounds	Bioactivities	Tax ID	Species Group Flag
CHEMBL1795087	Ubiquitin carboxyl-terminal hydrolase 1	O94782	SINGLE PROTEIN	Homo sapiens	22293	22528	9606	FALSE
CHEMBL5391	DNA polymerase iota	Q9UNA4	SINGLE PROTEIN	Homo sapiens	115089	116820	9606	FALSE
CHEMBL1075138	Tyrosyl-DNA phosphodiesterase 1	Q9NUW8	SINGLE PROTEIN	Homo sapiens	196426	345107	9606	FALSE
CHEMBL2146302	Glutaminase kidney isoform, mitochondrial	O94925	SINGLE PROTEIN	Homo sapiens	16265	16437	9606	FALSE
CHEMBL2288	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1	Q13526	SINGLE PROTEIN	Homo sapiens	35989	36236	9606	FALSE
CHEMBL3837	Cathepsin L	P07711	SINGLE PROTEIN	Homo sapiens	2763	3081	9606	FALSE
CHEMBL1075189	Pyruvate kinase isozymes M1/M2	P14618	SINGLE PROTEIN	Homo sapiens	6901	14432	9606	FALSE
CHEMBL2608	Lysosomal alpha-glucosidase	P10253	SINGLE PROTEIN	Homo sapiens	22273	35587	9606	FALSE
CHEMBL3577	Aldehyde dehydrogenase 1A1	P00352	SINGLE PROTEIN	Homo sapiens	75706	76445	9606	FALSE

The table presents a set of human protein targets retrieved from the ChEMBL database, along with their molecular identity, organism specificity, and associated bioactivity data. All listed targets are classified as single proteins from Homo sapiens (Tax ID 9606), confirming their direct relevance to human biological systems. The large number of associated compounds and bioactivities for each target indicates that these proteins are well studied and pharmacologically significant.

Several targets, such as ubiquitin carboxyl-terminal hydrolase 1, DNA polymerase iota, and tyrosyl-DNA phosphodiesterase 1, are involved in DNA repair, protein degradation, and genome stability, highlighting potential relevance to cancer progression and cellular stress responses. Metabolic enzymes including glutaminase (kidney isoform), pyruvate kinase M1/M2, and aldehyde dehydrogenase 1A1 play critical roles in energy metabolism and redox balance, processes that are often dysregulated in cancer and metabolic disorders. The presence of cathepsin L, a lysosomal protease, suggests possible involvement in extracellular matrix remodeling, invasion, and apoptosis. Overall, the diversity of targets and the high number of reported compounds and bioactivities indicate that the studied compound(s) may interact with multiple biologically relevant pathways, supporting a multi-target pharmacological profile with potential therapeutic implications in cancer and metabolism-related diseases.

CONCLUSION

This chapter focused on Schiff base complexes derived from 4-bromoaniline and salicylaldehyde coordinated with Fe(II), Cu(II), Zn(II), Cd(II), and Hg(II) ions. Characterization results supported coordination through azomethine nitrogen and phenolic oxygen atoms, forming stable metal complexes. Antioxidant studies, including nitric oxide and DPPH radical scavenging assays, revealed moderate activity across the series, consistent with trends reported for halogen-substituted Schiff base complexes. Protein-binding studies indicated meaningful interaction with biomolecules, suggesting potential biological relevance. The results underline the role of halogen substitution in influencing lipophilicity and biomolecular interactions.

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