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SYNTHESIS, MOLECULAR DOCKING, ANTIOXIDANT AND ANTICANCERACTIVITIESOF TETRAAZA MACROCYCLIC COPPER (II) COMPLEXES

Richa Kothari[1,*] and Anurag Agrawal[2]
[1]Department of Chemistry; School of Science, ITM University, Gwalior, (M.P) India-474005
[2.]School of Pharmacy, ITM University, Gwalior, (M.P.) India, 474005
Corresponding author: Richa Kothari
*E-mail: richakothari70@gmail.com

ABSTRACT

Three novel copper (II) complexes were synthesized from macrocyclic Schiff base ligand, which was derived from malonanilic acid hydrazone and thiosemicarbazide hydrochloride in 1:1 molar ratio. Schiff base ligand and all copper complexes were characterized by various physico-chemical and spectroscopic techniques like melting point, elemental analysis, molar conductance, UV-Vis, FT-IR, XRD, and thermo-gravimetric analysis (TGA). Spectroscopic studies confirm the distorted octahedral geometry of all the copper complexes. The biological activities of all compounds were evaluated like in-vitro antioxidant activity or percentage free radical scavenging effect via DPPH method against standard ascorbic acid and in vitro anticancer activity via MTT assay against MCF-7 breast cancer cell lines. Furthermore, for identification of binding modes of copper (II) complexes in the active pocket of target enzyme Topoisomerase IIα, molecular docking studies were also performed. Results of the biological activities showed that complex 1 exhibited the highest anti-cancer activity against MCF-7 cell line i.e. 7.21 ± 0.1 µg/ml among other copper complexes whereas compound 3 showed best antioxidant activity against ascorbic acid i.e. 86.04 µg/ml. Molecular docking study indicated here that all copper complexes were fitted into the active site of target enzyme and Copper complex 3 showed the maximum binding affinity (-20.4 kcal/mol) comparable to the other complexes.

Keywords: Synthesis, Tetraaza Macrocyclic Ligands, Copper(II) Complexes, Spectroscopic Study, Anticancer, Antioxidant Activities.

INTRODUCTION

After the successful application of Cisplatin drug as an antitumor drug, scientists across the globe, began interdisciplinary research on transition metal complexes for interaction with nucleic acids, carbohydrate, lipids and proteins as antitumor drugs[1,2] but the use of platinum metal in the synthesis of Cisplatin leads to serious side effects, which develops a challenge before researchers to overcome to prepare efficient anticancer drugs.[3,4] Nowadays medicinal inorganic chemistry offers vast possibilities for the design and development of metal-based novel drugs based on coordination behavior and redox properties of transition metal complexes to act as anticancer agents.[5,6]Recently a large number of metal complexes including Copper, Nickel, Lanthanum and Ruthenium complexes are developed as the most effective substitutes for classical Cisplatin type anticancer drugs.[7-17]

According to the classical concepts, Schiff bases have been considered as an important ligand due to their coordination behavior and they can be easily synthesized and coordinated with different kinds of metal ions in different geometries.[18] Due to the presence of an amine group similar to the natural biological system, ligands play a very important role in developing the conversion mechanism and racemization phenomenon in the natural biological systems.[19-25] Schiff base ligands have been used for various biological activities like anti-HIV, antitumor, anti-inflammatory, antimicrobial activities.[26-33] Among the various transition metal complexes, copper-based metal complexes have deeply been examined due to

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their low poisonous nature, low toxicity, and increased efficiency in terms of biological activities after the coordinating with Schiff base ligands.

In recent years scientists have shown great interest in the synthesis of copper-based complexes owing to their wide applicability in nucleic acid interactions and anticancer activity. In the present investigation, a hexadentate Schiff base ligand and its novel copper (II) complexes were successfully synthesized by easy one-pot chemical template synthesis and successfully characterized by various analytical techniques including UV-Visible, FT-IR, and XRD spectroscopy. Moreover, their Invitro anticancer activity was evaluated using MCF-7 cell lines. Also, antioxidant activity was performed via DPPH method against ascorbic acid. Molecular docking studies were carried out to find out the binding modes of copper complexes in the active pocket of target enzyme Topoisomerase IIα and to justify the rationales for their activities with the help of prerequisite interactions with Topoisomerase IIα enzyme.

EXPERIMENTAL

Materials and Methods

4-chloroacetophenone and 4-methyl aniline were produced from Sigma-Aldrich. Thiosemicarbazide hydrochlorides, Diethylmalonate were obtained from E. Merk Pvt. Ltd. All other general chemicals used in the present study were of high purity. Two solvents used were either spectroscopic pure or purified by the recommended method.[34]

Physical Measurements

Elemental analysisof complexes was determined in the Microanalytical unit using CHN elemental analyzer Perkin Elmer 2400 from CDRI Lucknow. FT-IR spectra of complexes were recorded on a Perkin Elmer FT-IR spectrometer in PC Ray research center ITM University, Gwalior. UV- VIS spectra of complex were recorded on a Perkin- Elmer UV/VIS Lambda 25 in PC Ray research center, ITM University, Gwalior. X-ray diffraction (XRD) analysis of complexes was carried out at room temperature using a Bruker axis D8 using Cu α radiation (λ =1.540 Å) over a 2θ collection range of 20 -80^{o} C and molar conductance of complexes were measured using a Digital conductivity meter in DMF solvent. All chemical reactions were monitored by TLC using pre coated aluminum sheets silica gel Merck 60F 254 and were visualized under UV Lamp. IUPAC nomenclature and calculation of molecular weights of complexes were performed by Chem Bio Draw I2 software.

Template Synthesis of Macrocyclic Copper Complexes

In the present study, Copper complexes were synthesized by template synthesis method. In template reactions, macrocyclic ligands are generated in the presence of transition metal ions. Briefly alcoholic solutions of copper salt mix in a stoichiometric solution of malonanilic acid hydrazones and thiosemicarbazide hydrochloride in 1:2: 2 molar ratios. The reaction mixture was refluxed for 8 hours with continuous stirring. After 8 hours refluxing, a brown precipitate appeared. The solvent was evaporated under reduced pressure and the residual obtained was quenched with ethanol. The obtained colored solid precipitates were filtered off, washed several times with cold ethanol and dried over fused $Cacl_2$ in desiccator. A good yield of products was obtained and the purity of the complexes was confirmed by the TLC and elemental analysis.

Molecular Docking

The three-dimensional crystallized structure of Human Topoisomerase IIα, PDB id: 4FM9) was retrieved from the protein data bank. For the docking process, optimization of a macromolecule (target protein) is a prerequisite step that involves the removal of water, native ligand and other heteroatoms that might be responsible for interruption in the molecular docking process. The structures of synthesized ligands were prepared by using ChemSketch (ACD 2012) and further saved as in .mol format after optimization in the three-dimensional structures and as per the compatibility of AutoDock Vina, .mol format is then converted into .pdb format through Open Bable -2.3.2. Subsequently, the synthesized ligands were loaded and their torsions along with rotatable bonds are assigned and the files are saved as ligand.pdbqt. In this study, the binding

modes of copper complexes with receptors were identified using AutoDock Vina software program. 1n this way, the 9 different conformers were generated of the compounds and blind docking was performed to confirm the actual binding site of the copper complexes on the molecular target and the best conformers in terms of binding affinity towards the target and appropriate interactions mandatory to inhibition of target enzyme particularly were discussed which might pave the way to disclose the mode of actions of synthetic ligands. The docking parameters were defined as coordinates of the center of binding site with X= 33.553, Y= 30.455, Z = 15.9l7 and binding radius = 1.000 and the grid dimension used for all the three (3) proteins are 47.25 x 47.25 x 47.25 A (grid size) with point separated by 1.000 A (grid- point spacing).The exhaustiveness (n= 24) was set for all the docking runs. The search algorithm was employed in the AutoDock Vina program to compute the binding energy of ligands to the enzyme.

In-vitro DPPH Radical Scavenging Activity of Complexes

The radical scavenging activity of complexes was determined by using DPPH assay according to Chang et al., (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as a reference.

Principle: 1,1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$(DPPH) + (H\text{-}A) = DPPH\text{-}A + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as a consequence, the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent Preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Working Procedure

Different volumes (2 - 20µl) of copper complexes were made up to 40 µl with DMSO and 2.96 ml DPPH (0.1 mM) solution was added. The reaction mixture was incubated in dark conditions at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the copper complexes was calculated using the following formula,

$$\%RSA = Abs\ control - Abs\ sample\ /\ Abs\ control \times 100$$

Where,*RSA* is the Radical Scavenging Activity; *Abs control* is the absorbance of DPPH radical + ethanol; *Abs sample* is the absorbance of DPPH radical + copper complexes.

Evaluation of Cytotoxicity Activity of compounds

The in-vitro cytotoxicity was performed for compounds on breast cancer cell lines MCF-7 to find a toxic concentration of the compounds by MTT assay.

Preparation of Compounds Solution

10mg of compounds was separately dissolved in 100ul of DMSO and volume was made up with MEM supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/ml concentration and sterilized by 0.22µl syringe filtration. Serial two-fold dilutions were prepared from the stock solution for future studies

Cell Line and Culture Medium

MCF-7 breast cancer cell lines were obtained from National Center for Cell Sciences (NCCS, Pune, India) and were cultured in MEM media supplemented with 10% inactivated Fetal Bovine Serum (FBS),

Penicillin (100 IU/ml),Streptomycin (100ug/ml) and Amphotericin B (5µg/ml) in a humidified atmosphere of 5% CO_2 at 37 ^{0}C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02%EDTA, 0.05% glucose in PBS). The cell cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 well microtitre plates (Tansons India Pvt. Ltd. Kolkata, India).

Cytotoxicity Studies

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells /ml using respective media viz., MEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension was added. After 24 hrs, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100ul of different concentration of compounds was added. The plate was then incubated at 37 ^{0}C for 72 hrs in 5% CO_2 atmosphere and microscopic examination was noted for every 24 hrs time interval.

MTT Assay

The cell viability is assessed by MTT reduction assay. After 72 hr of incubation, the compound solutions in the wells were discarded and 50µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 hrs at 37 ^{0}C in a 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plate was gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The plates were protected from light throughout the procedure. The percentage growth inhibition was calculated using the standard formula and concentration of compounds, needed to inhibit the growth of the cell by 50% i.e. CTC_{50} values were generated from the dose-response curves. The inhibition was expressed as the percentage relative to the cell control.

RESULTS AND DISCUSSION

This research work reports the synthesis, characterization of three novel Copper (II) complexes via the template method. All compounds were stable at room temperature and all are soluble in di methylformamide (DMF) solutions in all ratios. The elemental analyses of complexes were consistent with the proposed structure of the complexes. The molar conductance values of copper complexes were 29.3, 19 and 19.3 ohm^{-1} cm^2 mol^{-1} in DMF solution at room temperature confirms the formation of 1:2 electrolytic nature and indicates the non-electrolytic nature of copper complexes.

Table-1: Physical, Analytical and Electronic Spectral Data of Macrocyclic Cu (II) Complexes

Comp. No.	Cu(II) Compound/Molecular Formula	λ_M	µeff (B.M.)	λ_{max} (cm^{-1})	Analyses (%) Found (Calculated)				
					C	H	N	S	Cu
1.	$[Cu(C_{50}O_8H_{44}N_{16}S_2)].2H_2O$	29.3	1.94	37913, 40544	62.49 (61.23)	3.48 (3.20)	22.06 (21.42)	10.16 (10.19)	12.46 (12.49)
2.	$[Cu(C_{47}O_8H_{40}N_{16}S_2)].2H_2O$	19	1.98	36272, 34353	60.45 (59.3)	3.24 (3.45)	18.24 (18.28)	10.22 (10.20)	12.29 (12.36)
.3.	$[Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$	19.3	1.94	41430, 37249	61.63 (61.68)	3.16 (3.21)	12.24 (12.28)	10.20 (10.18)	12.46 (12.50)

Fourier Transform Infrared Spectral Analysis

This is a very reliable and accurate spectroscopic method to characterize the presence of various functional groups in synthesized compounds that are responsible for the formation of coordinate bonds with central metal ions. In IR Spectra, the presence of a strong bond at 1615-1626 cm^{-1} indicates the formation of C=N functional group in the *in-situ* reaction of template synthesis of the Copper complexes (28-29). The IR Spectra also show a low-intensity band in the region of 604-618 cm^{-1} indicates the formation of a coordinate bond between C=N functional group and Cu^{2+} .

Table-2: Infrared Spectral Data of Macrocyclic Cu(II) Complexes

S. No.	Compound	Selected IR Bands (cm^{-1})			
		$\upsilon_{(C=N)}$	$\upsilon_{(NH)}$	$\upsilon_{(Cu-N)}$	$\upsilon_{(C-S)}$
1	$Cu(C_{50}O_8H_{44}N_{16}S_2)].2H_2O$	1626.80	3320	618	760

2	$Cu(C_{47}O_8H_{40}N_{16}S_2)].2H_2O$	1620.10	3435.70	604.99	745
3	$[Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$	1615.43	3429.0666	610	790

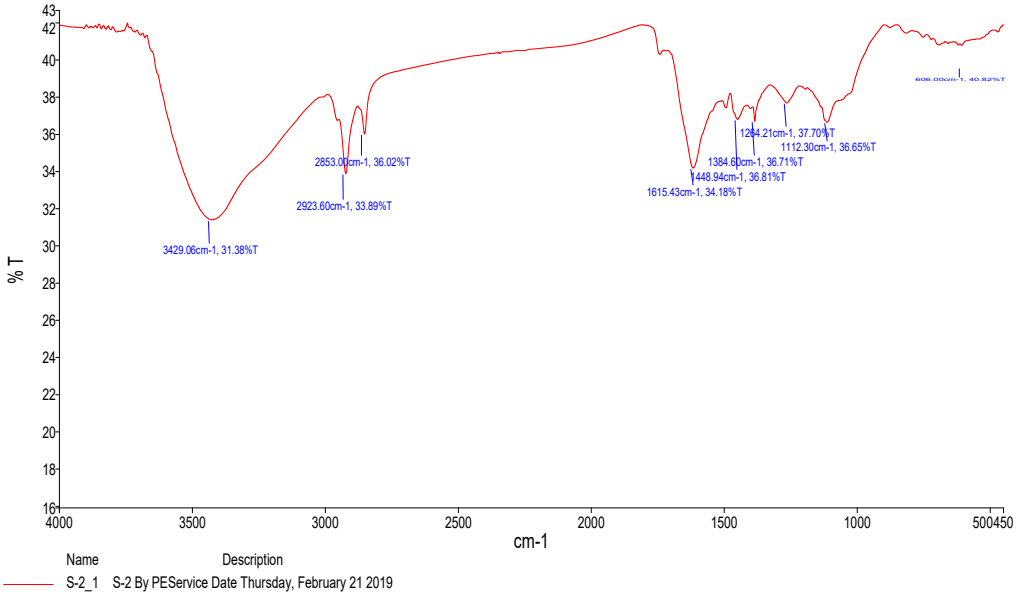

Fig.-1: FT-IR of $[Cu(C_{50}O_8H_{44}N_{16}S_2)].2H_2O$ Complex

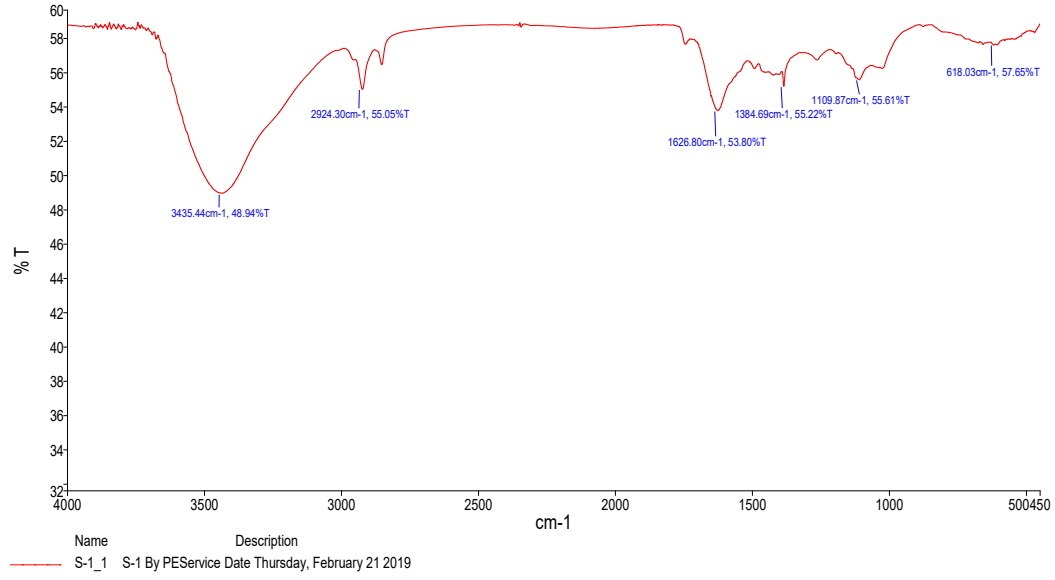

Fig.-2: FT- IR of $[Cu(C_{47}O_8H_{40}N_{16}S_2)].2H_2O$ Complex

Electronic and Magnetic Spectral Analysis of Copper (II) Complexes

The electronic spectra of Copper(II) complexes shows two strong absorption bands in the region of 40544- 34353 cm^{-1} and 41430- 37913 cm^{-1} due to $^2B_{1g}\rightarrow{}^2E_g$ and $^2B_{1g}\rightarrow{}^2A_{1g}$ electronic transitions. According to John- Teller distortion, a weak band due to $^2B_{1g}\rightarrow{}^2B_{2g}$ electronic transition is not observed separately due to the formation of distorted octahedral geometry of Copper (II) complexes. The magnetic moment of synthesized Copper (II) complexes at room temperature lie in the 1.86-1.90 Bohr magneton due to the presence of unpaired e^- in the 3d orbital of Cu^{2+} ion. The value of the magnetic moment of Copper(II) complexes indicates that they are monomeric in nature and there is no Cu – Cu interaction.

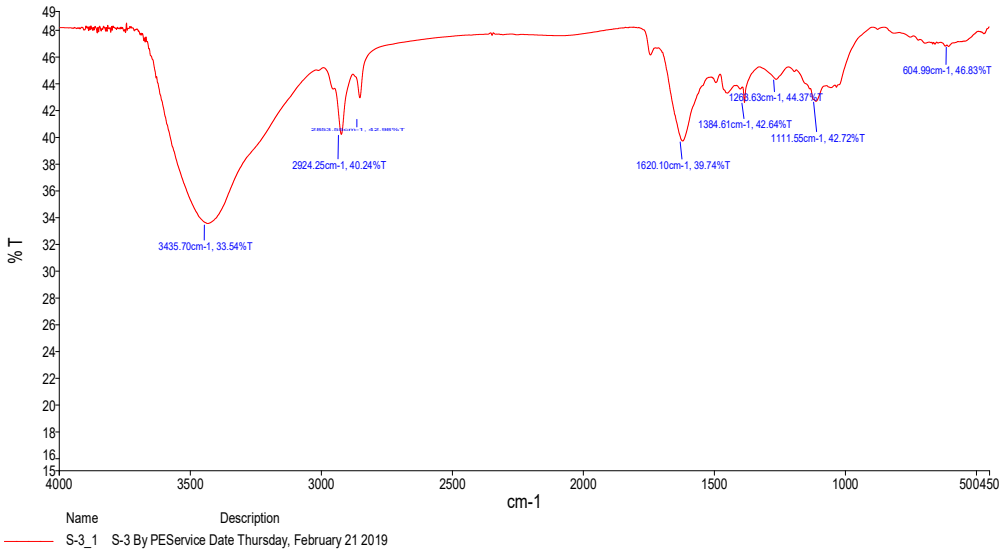

Fig.-3: FT- IR of $[Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$ Complex

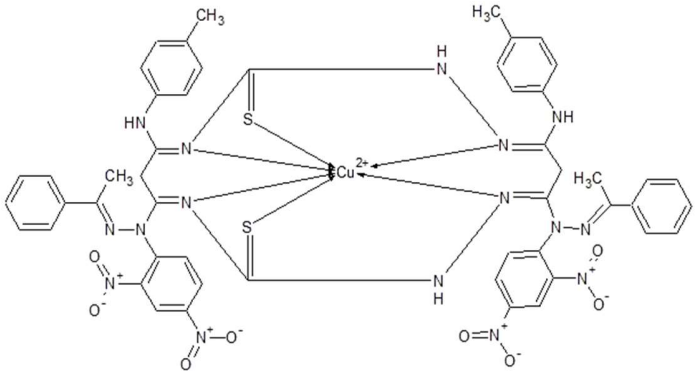

Fig.-4: Structure of $[Cu(C_{50}O_8H_{44}N_{16}S_2)].2H_2O$

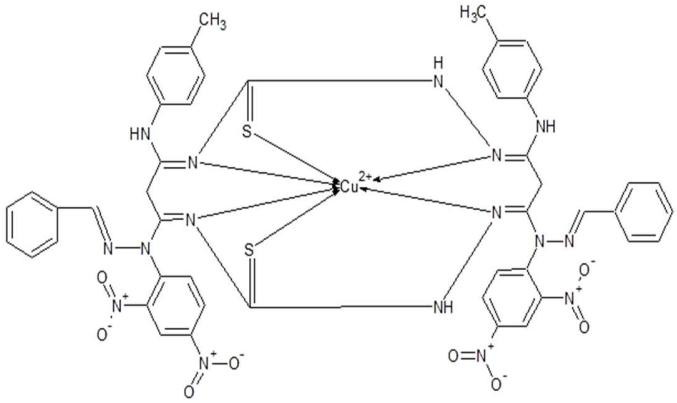

Fig.-5: Structure of $[Cu(C_{47}O_8H_{40}N_{16}S_2)].2H_2O$

Thermal Analysis

Thermal gravimetric analysis (TGA) and differential thermal stability of the complexes. The thermal behavior of all the copper complexes was studied in the range of 0 to 1400°C and it has been studied to establish different decomposition processes and to confirm the proposed stoichiometry. The results of such analysis have been summarized in Table-3 indicating a good correlation between calculated and found weight loss values.

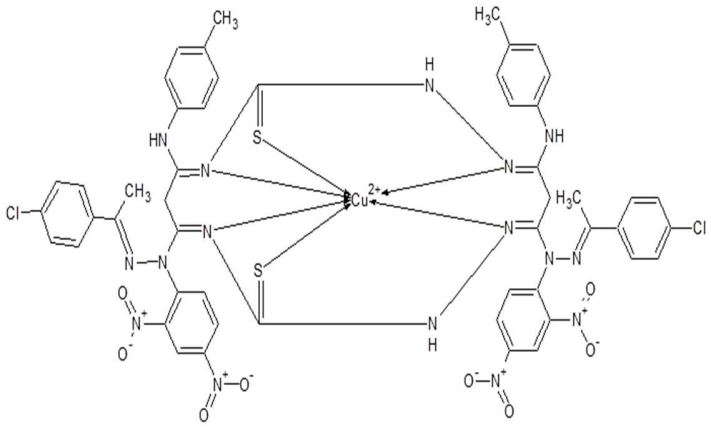

Fig.-6: Structure of $[Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$ Complex

Thermal analysis plays an important role in analyzing the stability melting point, structure and decomposition properties of the metal complexes. TGA was studied from room temperature to 1400° C in an inert nitrogen atmosphere. TGA curve was drawn a percentage mass loss Vs temperature. The TGA decomposition curves of complexes showed peaks in the temperature range of 142.18°C-313.49° C, 76.85° C-242.88° C and 134.41°C-345.82° C due to loss of organic species.

All complexes showed initial weight loss at 10.34 % (2.473 mg), 11.04 % (2.7116 mg) and 72.14 % (10.50 mg) respectively due to loss of organic species attached to Copper (II) ion. The overall mass loss observed was 93.52 % and it was compound with theoretical mass loss value which was found correct. The end Product estimated as CuO. The observed mass and the calculated mass were almost equal.

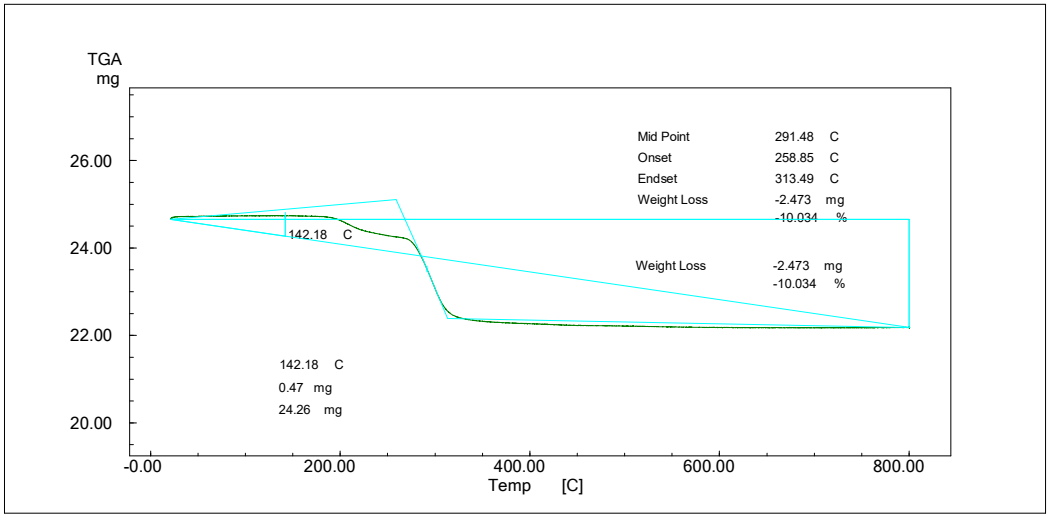

Fig.-7: TGA Spectra of $[Cu(C_{50}O_8H_{44}N_{16}S_2)].2H_2O$

Powdered XRD Studies

The XRD pattern of synthesized Copper(II) complexes (Figures 10,11,12)showed well defined crystalline peaks indicating that the complexes were amorphous. It has specific 'd' value which can be used for the size of the complexes d_{XRD} could be estimated from XRD patterns using Scherre's formula:

$$d_{XRD} = \frac{0.9\lambda}{\beta . \cos \theta}$$

Where λ = is the wavelength, β= is the full width at half maxima Θ= is the diffraction angle using the full width at half maximum intensity of XRD patterns, the average particle size of Copper complexes was 3.86, 7.37, 0.45 nm. The particle size of all Copper complexes was in the diameter range of nanosize.

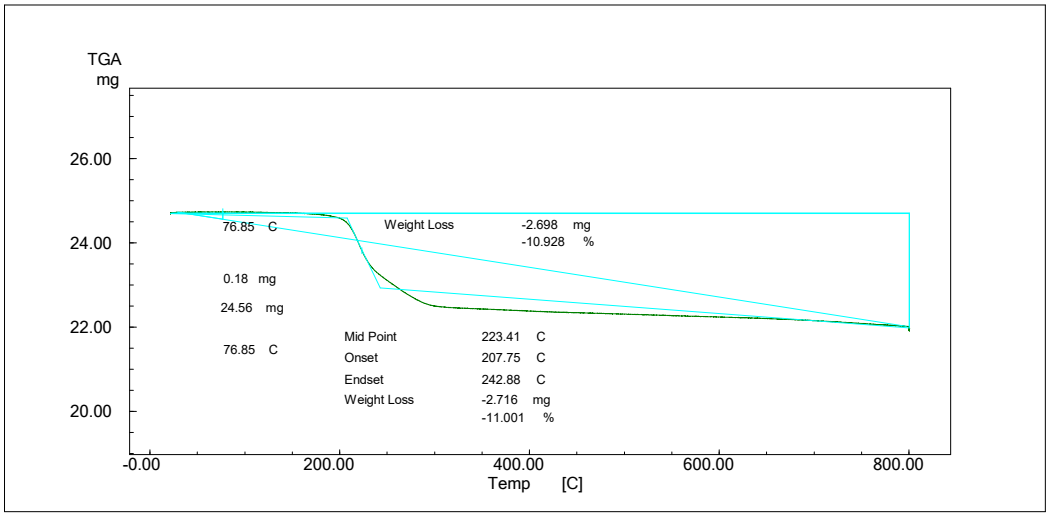

Fig.-8: TGA Curve of $[Cu(C_{47}O_8H_{40}N_{16}S_2)].2H_2O$ Complex

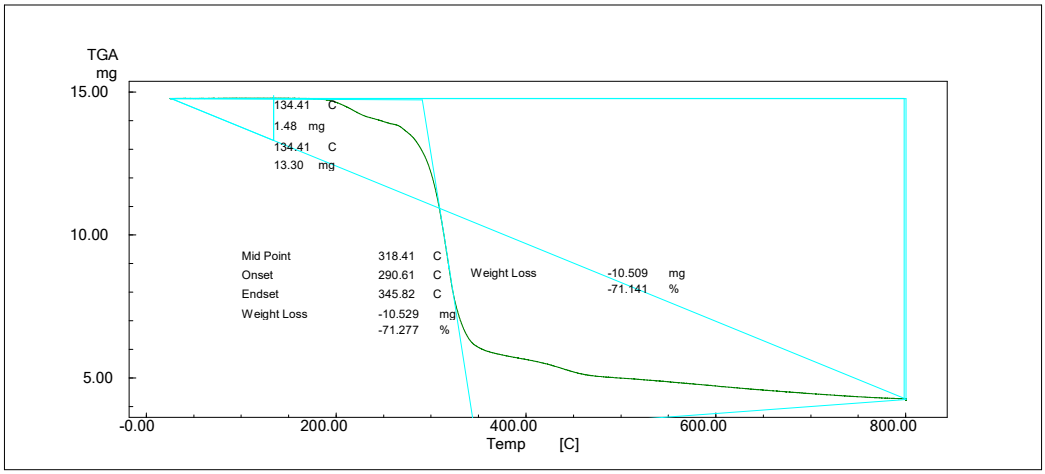

Fig.-9: TGA Curve of $[Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$ Complex

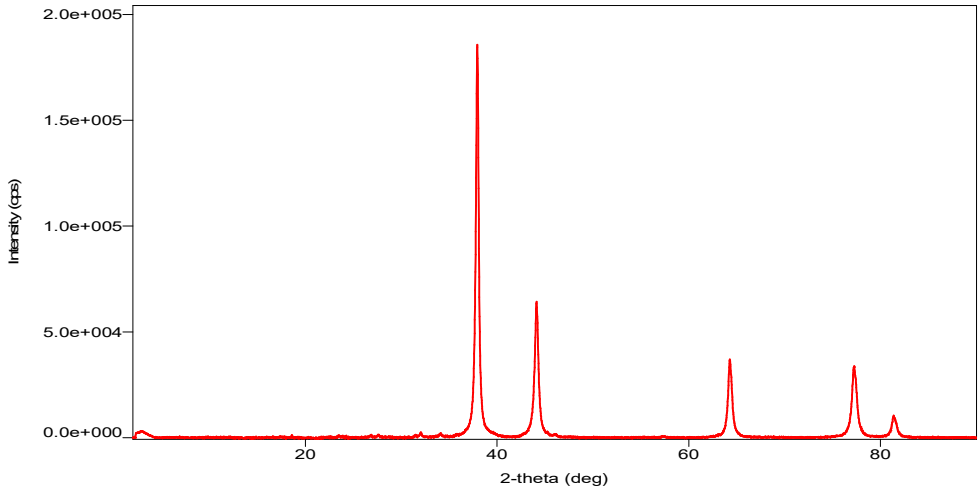

Fig.-10: XRD Spectra of $[Cu(C_{50}O_8H_{44}N_{16}S_2)].2H_2O$

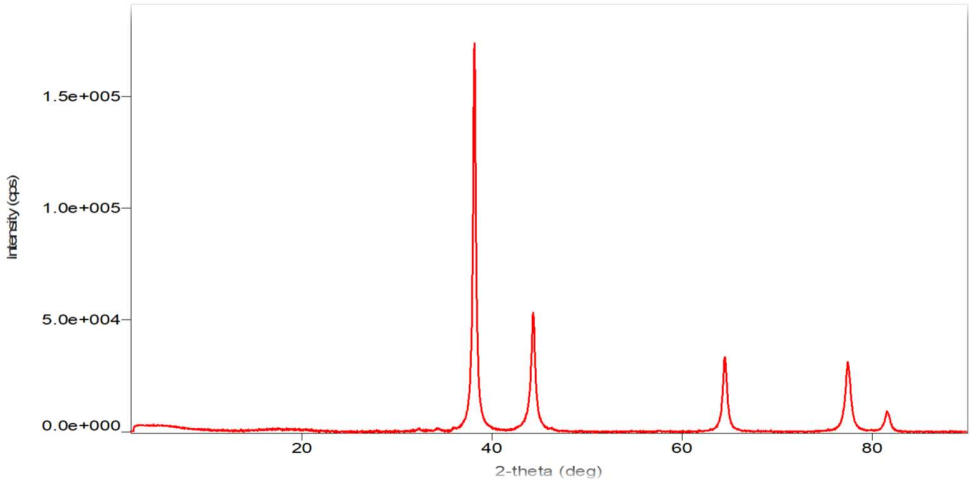

Fig.-11: XRD Spectra of $[Cu(C_{47}O_8H_{40}N_{16}S_2)].2H_2O$

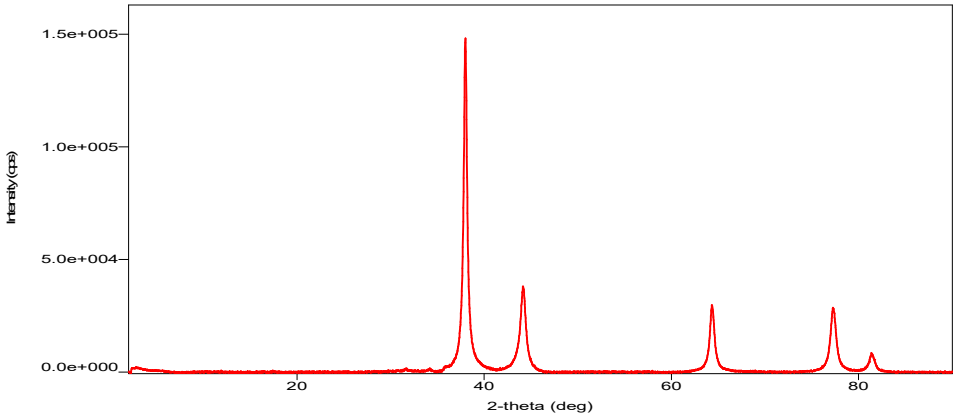

Fig.-12: XRD Spectra of $Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$

In-vitro Antioxidant Activity of Copper Complexes via DPPH Assay

With the Pairing of electrons in stable radical DPPH after reacting with the reducing agent, it loses its pink color stoichiometrically depending on the number of electrons accepted by DPPH radical by the antioxidant or reducing agent. The loss of color due to the reaction with Copper complexes and standard antioxidant ascorbic acid is the indicator of their antioxidant potential which could be measured using a UV-VIS spectrophotometer.[35]

The DPPH scavenging activities of Copper complexes were expressed as IC_{50}, whose concentration is sufficient to obtain 50 % of maximum scavenging activity. The IC_{50} values of Copper complexes are presented in Table-4. In this experiment, ascorbic acid was used as a standard compound. The results clearly showed that the complex $[Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$ showed the highest antioxidant activity as compared to other Copper complexes. The DPPH activity of Complexes depends on the presence of various functional groups like hydroxyl, carbonyl, imine groups in complexes as well as on the geometry of complexes. The order of antioxidant activity of Copper complexes is as follows 3 > 1 > 2 according to their IC_{50} values against standard ascorbic acid.

In-vitro Cytotoxic Activities of Synthesized Cu(II) Complexes

Invitrocytotoxic activity of copper complexes was tested against human breast cancer cell line (MCF-7) by MTT assay cells were exposed to a different concentration ranging from 1000 µg/ml to 7.8 µg/ml to determine the percentage growth inhibition on MCF-7 breast cancer cell lines.

Table-3: IC_{50} Values of DPPH Radical Scavenging Activity of Cu(II) Complexes

S. No.	COMPOUND	IC_{50} µg/ml
1.	$[Cu(C_{50}O_8H_{44}N_{16}S_2)].2H_2O$	260.95 ± 0.66
2.	$[Cu(C_{47}O_8H_{40}N_{16}S_2)].2H_2O$	263.82 ± 2.38
3.	$[Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$	86.04 ± 1.57
4.	Ascorbic acid	21.63± 0.33

Table-4: IC_{50} Values of Anticancer Activity of Cu (II) Complexes

S. No.	Compounds	IC_{50}(µg/ml)
1.	$[Cu(C_{50}O_8H_{44}N_{16}S_2)].2H_2O$	7.21 ± 0.1
2.	$[Cu(C_{47}O_8H_{40}N_{16}S_2)].2H_2O$	9.29 ± 0.1
3.	$[Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$	13.01 ± 0.2
4	Cisplatin	1.9

The cytotoxic activity of Copper complexes proved that all complexes were showed considerably excellent activity as compared to the standard drug Cisplatin. The cytotoxic activities of Copper complexes (IC_{50} values) were in the range of 7.21 ± 0.1 to 13.01 ± 0.3 µg/ml. Results showed that the first complex has the lowest cytotoxic activity (IC_{50} = 7.1 ± 0.1 µg/ml) as compared to other Copper complexes. The standard Cisplatin drug has cytotoxic activity 1.9 µg/ml against MCF-7 breast cancer cell lines. The result of the cytotoxic activity of Copper complexes revealed that the first Copper complex expressed significant cytotoxic potency and can be considered as a new lead compound for further study.

Molecular Docking Studies of Synthesized Copper (II) Complexes

The docking results indicate that all copper complexes were found to be fitted into the active site of the enzyme and copper complex III has maximum binding energy (-20.4 kcal/ mol) among others and made hydrogen bond with residue Arg713 of the enzyme. Copper complex1 could not afford any hydrogen bond with the active site of the target enzyme but have a significant binding affinity (-19.8 kcal/ mol). Copper complex II made three hydrogen bonds with different residues i.e. Arg661 and Gly859 of the enzyme with a good binding affinity of -19.5 kcal/ mol. Copper complex I also formed a hydrogen bond with Arg929 but binding affinity is least among other complexes. These results reveal here that a significant binding affinity of copper complexes responsible for the anti-cancer activity in humans. Different amino acid interactions obtained through molecular docking study also suggest that these copper complexes would be less toxic than those conventional drugs used in the treatment of malignancy and also pave the way for more preclinical and clinical experimentations.

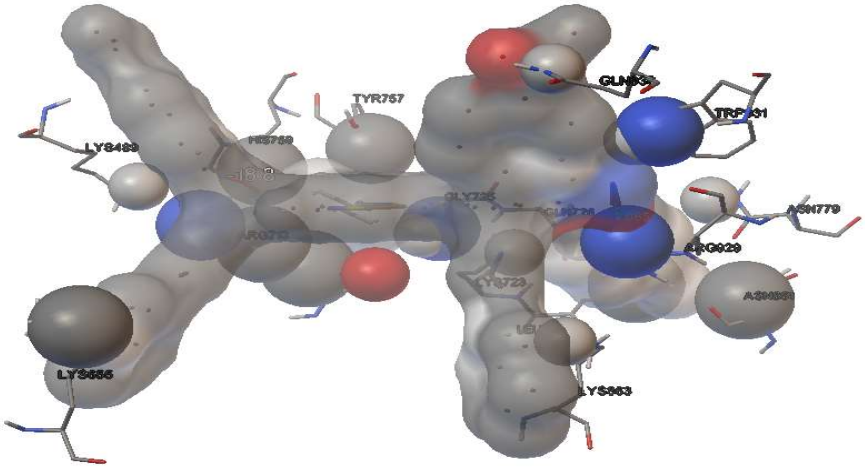

Fig.-13: A Binding Mode of Copper Complex1 with topoisomerase IIα
(3D Model of Interactions between Ligand and Target)

Fig.-14: A Binding Mode of Copper Complex2 with Topoisomerase IIα
(3D Model of Interactions between Ligand and Target)

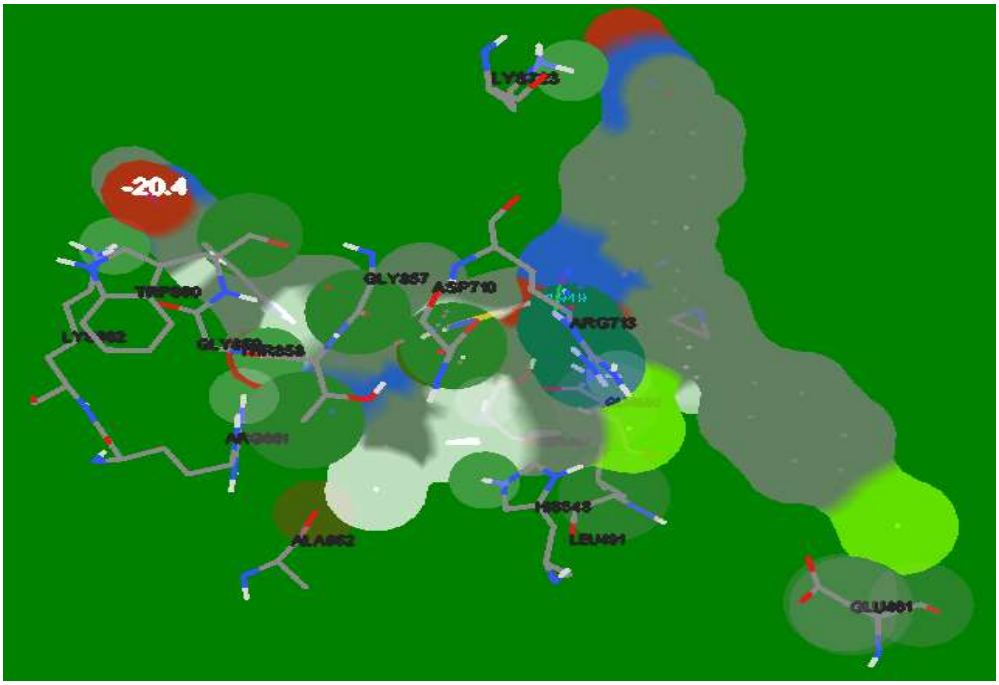

Fig.-15: A Binding Mode of Copper Complex3 with Topoisomerase IIα
(3D Model of Interactions between Ligand and Target)

Table-5: Docking Studies of Cu (II) Complexes

S.No.	Ligand_Receptor	Binding Affinity (-Kcal/mol)	Amino Acids involved in interactions	No. of H-Bond (Formed between)	H-Bond Distance (Å)
1.	$[Cu(C_{50}O_8H_{44}N_{16}S_2)].2H_2O$	18.8	Arg713, His759, Tyr757, Gly725, Lys723, Lys863, Gln726, Arg929, Asn779, Trp931, Leu722, Lys489, Gln938, Lys655, Asn779	01(4fm9:A:ARG929:NH1,cu2_out)	2.065

2.	$[Cu(C_{47}O_8H_{40}N_{16}S_2)].2H_2O$	19.5	Thr858, Arg661, Gly857, Asp710, Ser497, Lys489, Thr757, Phe653, Ser861, Thr767, Lys489	03(4fm9:A:ARG661:NE,Cu3_out, 4fm9:A:ARG661:NH2,Cu3_out, 4fm9:A:GLY859:,Cu3_out)	1.82, 2.121, 1.866
3.	$[Cu(C_{50}O_8H_{42}N_{16}S_2)].2H_2O$	20.4	Leu491, His548, Arg713, Asp710, Lys723, Gly857, Ala652, Thr858, Arg661, Lys662, Gly559, Trp860, Glu461, Gln500, Ser497	01(4fm9:A:ARG713:NE,cu4_out)	1.943

CONCLUSION

In the present study, we have prepared a series of novel macrocyclic copper complexes and characterized them using various physico-chemical and spectroscopic techniques. Copper complexes showed excellenttomoderate cytotoxic activity against MCF-7 breast cancer cell line using Cisplatin a s standard drug. The docking results indicate that all copper complexes were found to be fitted into theactive site of the enzyme and copper complex 3 have maximum binding energy(-20.4kcal/mol) among other copper complexes and demonstrated the highest affinity towards target enzyme along with making a hydrogenbond with residueArg713oftheenzyme. Results showed that 3 copper complex showed more pronounced antioxidant activity in the presence of DPPH using ascorbic acid as a standard compound.The cytotoxicity screening of the copper complexes revealed that these complexes showed reasonable cytotoxic activity against MCF-7 breast cancer cell line in comparison to the traditional anticancer drug Cisplatin. According to cytotoxicity results, it was clear that 1 copper complex expressed significant cytotoxic potency and can be considered as a novel compound in the treatment of breast cancer.

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