IJPSR (2020), Volume 11, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 May 2019; received in revised form, 21 August 2019; accepted, 02 September 2019; published 01 February 2020

SYNTHESIS, IDENTIFICATION OF *IN-SILICO* INTERACTIONS AND BIOLOGICAL STUDIES OF NOVEL SCHIFF BASE LIGAND AND ITS COPPER (II) COMPLEX

Sanchita Rai [1], Anurag Agrawal [2] and Richa Kothari [* 1]

Department of Chemistry, School of Sciences [1], School of Pharmacy [2], ITM University, Gwalior - 474001, Madhya Pradesh, India.

Keywords:

Copper (II) complexes, Anti-cancer, Anti-oxidant, MCF-7 cell line, Molecular docking

Correspondence to Author:
Dr. Richa Kothari

Department of Chemistry, School of Sciences, ITM University, Gwalior - 474001, Madhya Pradesh, India.

E-mail: richakothari70@gmail.com

ABSTRACT: A novel copper (II) complex has been synthesized with Schiff base ligand obtained by the reaction of substituted carbohydrazone and thiosemicarbazide hydrochloride from the condensation reaction of ester and primary aromatic amine. The synthesized ligand and its copper (II) complex were characterized by various spectroscopic techniques like FT-IR, H1 NMR, UV-Visible, Fluorescence, powder XRD and Thermo-gravimetric analysis. Ligand and its copper complex were also screened for antioxidant and anticancer activities. In Cu (II) complex 1:2 copper to ligand molar ratio was obtained from analytical data. The molar conductance data confirm that complex is non-electrolytic in nature. Based on the electronic and magnetic data, a distorted octahedral geometry is ascribed for the Cu (II) complex. Ligand and its Cu (II) complex showed considerable anticancer activity against MCF-7 cell lines and more pronounced antioxidant activity in the presence of DPPH. Furthermore, molecular docking of ligand and its copper complex into the binding site of topoisomerase IIα was carried out using Auto Dock Vina software which demonstrated significant binding energies of copper (II) complex when compared to Schiff base ligand. The result of the docking study exhibited that copper complex shows-19.8 kcal/mol binding energy and fitted into the same active site where Schiff base ligand occupied with -8.9 kcal/mol binding energy.

INTRODUCTION: The chemistry of transition metal complexes has conferred considerable recognition largely due to their catalytic and bioinorganic relevance. These complexes are also important due to their potential biological activities such as antimicrobial, anti-viral, anti-fungal, anti-malarial and antitumor [1-6]. A transition metal ion can coordinate a ligand in a meticulous three-dimensional configuration thus enable the modification of the molecule to recognize and interact with a defined molecular target.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(2).745-54

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(2).745-54

This is further expanded by the variety of chemical modification of ligands and selection of metal ions. Transition metal ions possess different oxidation states which not only allow for modification of the three-dimensional space into which the molecule can fit but remarkably permit them to engage in biological redox chemistry [7-8].

Further, the capacity of the transition metal ion to undergo ligand exchange reactions offers unique characteristic for metal ions to interact and coordinate with biologically important ions and molecules. In recent years, great attempts have been made on the design, synthesis, and growth of new luminescent material for a great array of applications in sensor technology, electro-luminescent devices, photo molecular switches and dyes. Literature reveals that many of the transition metal complexes have been used as luminescent

markers. For the coordination of metal ion, ligand systems have been developed which combine good donor abilities with fluorescent properties [9]. Medicinal inorganic chemistry is comparatively a new discipline, which developed after the unique discovery of the anti-tumor activity of Cisplatin [10]. The clinical success of this complex has encouraged significant interest in the forage for new metal complexes as modern therapeutic, diagnostic and radiopharmaceutical agents. In this connection, copper and zinc complexes are used in the treatment of many diseases including cancer [11-13].

Cobalt complexes have been investigated as potential hypoxia-activated prodrugs [14-21], whereas chromium, manganese, and iron complexes have been used for antibacterial activity [22]. Zinc metal is necessary for the structure, regulation and catalytic action of over 300 enzymes [23-24]. Literature reveals that the biological properties of metals are determined both by speciation and the ligand present around the metal center. One of the most important approaches in anti-cancer chemistry is attentive on the design of new metal compounds with disparate substituents and labile sites which may raise their cytotoxicity specifically to cancer cells. According to literature; accessible redox states, thermodynamic and kinetic study, and the unique properties of the cationic central metal ion and the ligand itself present a wide range of reactivities that can be utilized. Recent literature also discloses that few Cu^{2+} complexes supported with azomethine based macrocyclic ligands containing labile sites were shown to have excellent anti-cancer properties. In spite of the enormous efforts in the discovery and expansion of new anticancer drugs, cancer still remains one of the leading causes of death worldwide [25].

One characteristic of cancer cells is their highly proliferative nature; consequently, retardation of proliferative pathways is contemplated to be a productive strategy to fight cancer. Much attention has recently been given to the discovery and growth of new, more selective anticancer agents that inhibit proliferative pathways of cancer cells [26-28]. The understanding of the mechanisms of cell-death execution and the role that they play in various different diseases opens new and wide therapeutic strategies. In this aspect, mammalian Topoisomerase II is one of the well-known targets for antitumor agents like doxorubicin, etoposide, ellipticine, and amsacrine [29]. Schiff base derivatives of copper complexes are familiar for their diverse biological activities which include antitumor, anti-inflammatory [30] and antimicrobial activity [31]. The present work is based on the continuation of our research for the synthesis of coordination complexes of copper metal and evaluating their biological activity. In this paper, we report the synthesis, characterization, molecular docking and biological activities of novel Schiff base ligand and it's copper (II) complex obtained by the reaction of substituted carbohydrazone and thiosemicarbazide hydrochloride from the condensation reaction of ester and primary aromatic amine.

MATERIALS AND METHODS: The reagents and chemicals were obtained from the commercial sources and used as such. Cinnamaldehyde, 2, 4 dinitrophenyl hydrazine, thiosemicarbazide hydrochloride, diethyl malonate, p-toludiene, Cu (II) chloride, DPPH, and solvents used were of purchased from Merck. Melting points were uncorrected and determined by the Electro-thermal IA 9100 melting point apparatus. Elemental analysis (C, H, N, and S) was performed using Perkin Elmer CHNS analyzer.

IR spectra of ligand and its Cu (II) complex were recorded on Perkin Elmer FTIR spectrophotometer within the range of 4000- 400 cm^{-1} using KBr disc, Molar conductance of the complex was measured using a Digisun conductivity meter in DMF (1×10^{-3} molar conc.). The electronic absorption spectra of the ligand and its Cu (II) complex were recorded using Perkin Elmer Spectrophotometer from 200 - 800 nm. The X-rays pattern of the complex was recorded on Xpert Pro X-ray diffractometer with Cu-K radiation (λ = 1.546Å). All synthetic reactions were monitored by TLC using precoated Aluminium sheet silica gel Merck 60F 254 and were visualized by the UV lamp.

Synthesis of Schiff Base Ligand: A novel Schiff base ligand was designed to offer coordination via S and Namine atoms in their protonated form. This coordination mode may result in the formation of the tridentate ligand. The ligand was synthesized by the condensation reaction between acid hydrazide and an aromatic aldehyde.

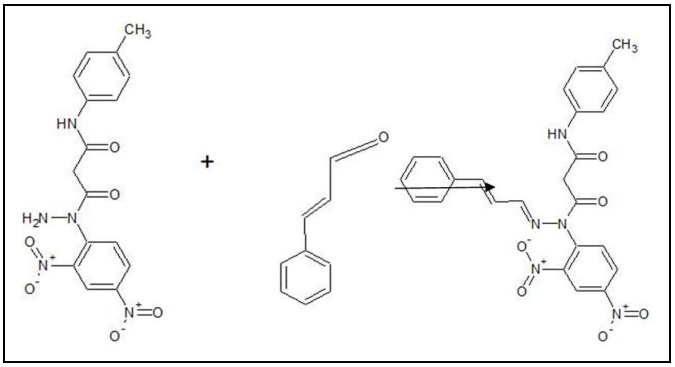

FIG. 1: SCHEME OF SYNTHESIS OF SCHIFF BASE LIGAND

Yield 63%, elemental analysis; C 72.72% (Calc. 73.42), H 4.50% (Calc. 4.42%), N 8.36% (Calc. 8.30%) SI- m/s (rel. Int. %) (mt), FTIR (KBr pellet, cm^{-1}): 2998.15 cm^{-1} (νC-H of C_6H_5); 997 cm^{-1}, 829 cm^{-1}, 742 cm^{-1} (C-H out of plane vibration for C_6H_5); 1335 (ν C-O); 1627 (C=O), 1591 (C=N); 1318 (C-N); 3343 (O-H), 1H NMR (300 MHz) $CDCl_3$; 8.8641 (S, HC=N), 8.394 (d, Ar-H), 8.4138 (S, HC=N), 7.9418 (d, Ar-H), 7.6704 (m, Ar-H), 7.9418 (m, Ar-H), 7.34 (m, Ar-H),

Synthesis of the Copper Complex of Novel Schiff Base Ligand: 0.1 ml of the ligand was dissolved in 10 ml of hot methanol, and 0.2 mol of $CuCl_2.2H_2O$ was added drop-wise with continuous stirring followed by further stirring for 6 h. Dark orange product was obtained which was filtered and washed with distilled water. The compound was recrystallized with ethanol.

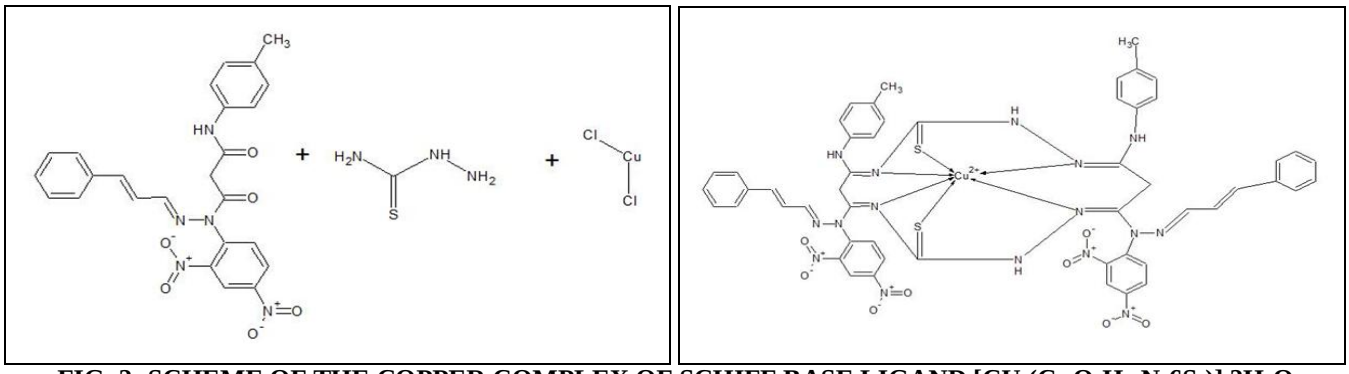

FIG. 2: SCHEME OF THE COPPER COMPLEX OF SCHIFF BASE LIGAND [CU ($C_{52}O_8H_{44}N_16S_2$)].$2H_2O$

Molecular Docking Study: The crystal structure of the molecular target (Human Topoisomerase IIα, PDB id: 4FM9) which is engaged in the development of different malignancy after some mutations, was retrieved from RCSB protein data bank [32]. Macromolecule has to be prepared, prior to docking process. Preparation involves removal of the water molecule and any unwanted heteroatoms, because these may interfere in the docking process. After refining, macromolecule was saved as pdb execution file. The macromolecule was loaded and stored as macromolecules. PDBQT after assigning hydrogen bonds and gasteiger charges.

Investigational ligands were designed using Chem Sketch (ACD 2012) and optimized for energy minimization using MM2 force field and saved in .mol format subsequently converted into .pdb format by Open Bable -2.3.2 software. The investigation ligand was 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 the copper complex with receptor was identified using Auto Dock Vina software program. In this way, the 9 different conformers were generated of the compounds and blind docking was performed to confirm actual binding site of the copper complex on the molecular target and the best conformers were discussed with lowest binding energy (-kcal/mol)which might pave the way to disclose the mode of actions of synthetic ligand.

The docking parameters were defined as coordinates of the centre of binding site with x = 33.553, y = 30.455, z = 15.917 and binding radius = 1.000 Å and The grid dimension used for all the three (3) proteins are 47.25 × 47.25 × 47.25 Å (grid size) with point separated by 1.000 Å (grid-point spacing). The exhaustiveness (n = 24) was set for all the docking runs. The search algorithm was employed in the Auto Dock Vina program to compute the binding energy of ligands to the enzyme. Conformers of the ligand were

automatically docked to the enzymes and most stable conformer in terms of binding affinity (most negative) was used for post-docking analysis [33].

DPPH Radical Scavenging Activity: The free radical scavenging activity of the ligand and its Cu (II) complex were determined by using DPPH free radical scavenging method. Compounds were dissolved in DMSO (1 mg/ml) and used as stock solutions. From the stock solutions, 0.01 ml of each compound solution with different concentration (2500 - 7.8 µg/ml compounds) was added to 0.2 ml of the methanolic solution of DPPH. Both tubes were incubated at 37 ºC for 30 min. After incubation 0.1 ml of the reaction mixture was pipette out to microlitre plate. Absorbance was measured at 490 nm using a microlitre reader. The same procedure was repeated for the standard by replacing test samples with standard test and control was performed in triplicate and test blank and control blank were conducted in a singlet. The percentage of scavenging activity of DPPH free radical was measured by using the following formula

$$\text{Scavenging activity (\%)} = (A_o - A_i) / A0$$

Where A_o is the absorbance of the control and A_i is the absorbance of the sample

Anticancer Activity: MCF- 7 Cell lines (human breast carcinoma cell line) were obtained from National Centre for Cell Science (NCCS), Pune, India. The cell lines were cultured in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), Amphotericin (µg/ml), Gentamycin (µg/ml), streptomycin (250 µg/ml) and penicillin (250 unit/ml) in a CO_2 incubator at 5% CO_2. About 700 cells/well were seeded in 96 well plates using culture medium. The viability of the cells was tested using trypan blue dye with the help of hemocytometer and 95% of viability was confirmed. After 24 h the new medium with the compound in the concentration of 125 µg/ml to 3.1 µg/ml was added at respective wells and kept in incubation for 48 h. After incubation, the following assay was performed.

MTT Assay: After 48 h of the compound treatment, the medium was changed again for all compounds and 10 µg of MTT (5 mg/ml stock solution) was added and the plates were incubated for an additional 4 h. The medium was discarded and the formazon blue, which was formed in the cells, was dissolved with 50 µl of DMSO. The optical density was measured at 570 nm. Cisplatin was used as a standard. The percentage of toxicity was calculated by using the following formula

Cytotoxicity = 1- ((O.D of treated cells) / (O.D of untreated cells))

The IC_{50} (concentration of drug required to inhibit the growth of 50% of the cancer cells) values of the compounds were calculated using the graph pad prism software tool.

RESULT AND DISCUSSION: The physical and analytical data of ligand and its Cu (II) complex are depicted in **Table 1**. Ligand and its copper complex was colored and very stable at room temperature, soluble in DMF and DMSO. Analytical data confirm the metal to ligand ratio is 1:2 in the copper complex. The molar conductance measurement of the complex was recorded in DMF (1×10^3). The results indicate the non-electrolytic nature of the complex.

TABLE 1: THE PHYSICAL AND ANALYTICAL DATA OF LIGAND AND ITS CU (II) COMPLEX

S. no.	Compound	Molecular weight (g/mol)	Colour	Yield %	Elemental analysis					Molecular conductivity $mho^{-1}mol^{-1}cm^2\lambda$
					C	H	N	S	Cu	
1	Schiff base $C_{52}O_8H_{44}N_{16}S_2$)	487	Red	69	60.6 (61.)	3.46 (3.400)	4.47 (450)	-	-	-
2	$[Cu(C_{52}O_8H_{44}N_{16}S_2)].2H_2O$	1194	Maroon	79.2%	64.1 (64.38)	3.53 (3.49)	4.49 (4.63)	10.28 (10.30)	10.12 (9.63)	34.1

UV- Visible Spectral Studies: The UV-Visible spectra were conducted in order to obtain the geometry of the complex. The UV-Visible spectra of ligand showed two well-defined peaks at 211 nm and 230 nm, due to π→π* and n→π* transitions respectively. **Fig. 3** & **4** show the electronic spectra of ligand and Cu (II) complex in DMSO. When ligand formed a complex with Cu (II), the peaks at 211.48, 230 nm disappeared and a new well-defined peak appeared at 247 nm. Formation of the

coordinate bond of the ligand with Cu (II) complex redistributed the electron densities of the compound. The 281 nm peak should be aroused due to the ligand to metal charge transfer. Another peak at 700 nm was observed due to d→d transition originating at Cu (II).

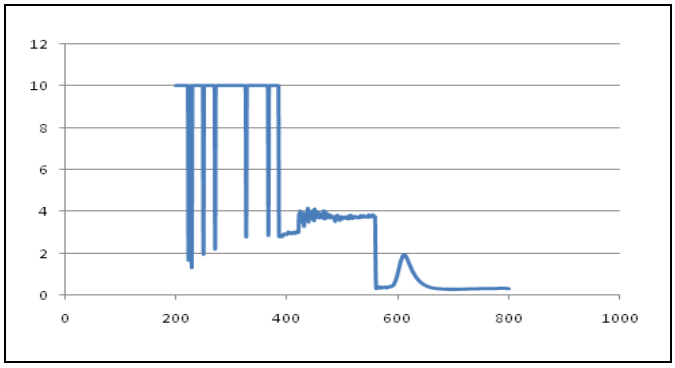

FIG. 3: ELECTRONIC SPECTRA OF SCHIFF BASE LIGAND

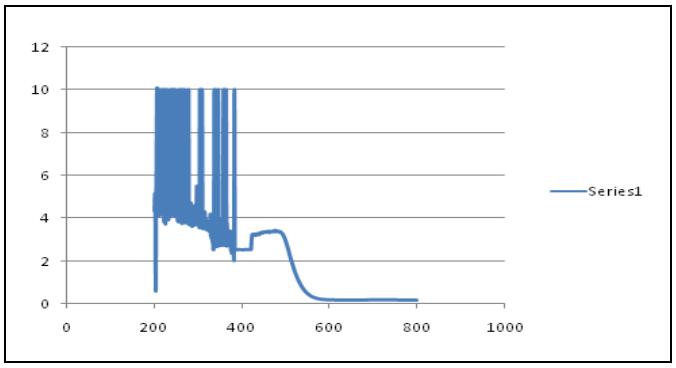

FIG. 4: ELECTRONIC SPECTRA OF THE COPPER COMPLEX OF SCHIFF BASE LIGAND

FTIR Spectral Studies: The FTIR spectral data containing relevant vibrational bands of the ligand and Cu (II) complex is listed in **Table 2**. The ligand showed a band in the range of 1615-1680 cm^{-1} which is due to (C=0) group of the hydrazide-hydrazone moiety, this band was shifted to lower wavenumber region 5-75 cm^{-1} in their corresponding Cu (II) complex, indicating the coordination of nitrogen atom of the ligand [34]. The stretching vibration of the azomethine group (C=N) was observed in the range of 1613 - 1590 cm^{-1} in the ligand. This band was shifted to 30 - 40 cm^{-1} lower wavenumber region in Cu (II) complex, indicating the participation of nitrogen atom of azomethine group in coordination to the metal ion participation of the C=S group in chelation is ascertained from the shift of (C-S) at 780.28 cm^{-1} in the ligand to the lower frequencies by 40 cm^{-1} in the complex [34]. Further, the coordination of nitrogen was supported by the appearance of a non-ligand band at 600 - 400 cm^{-1} region due to the (Cu-N), respectively. From the above spectral data, it was concluded that Schiff Base ligand acts as a monobasic tridentate ligand with NNS donor sites.

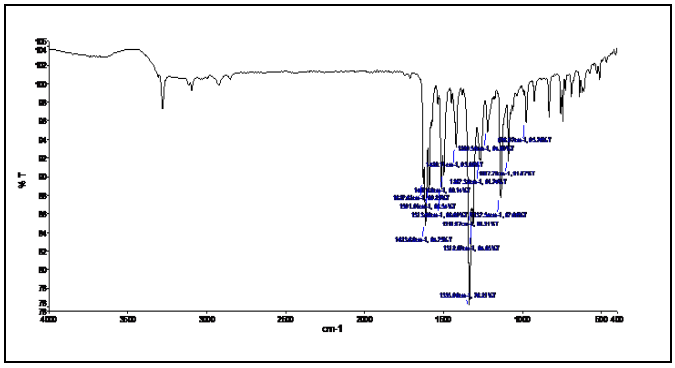

FIG. 5: FT-IR SPECTRA OF SCHIFF BASE LIGAND

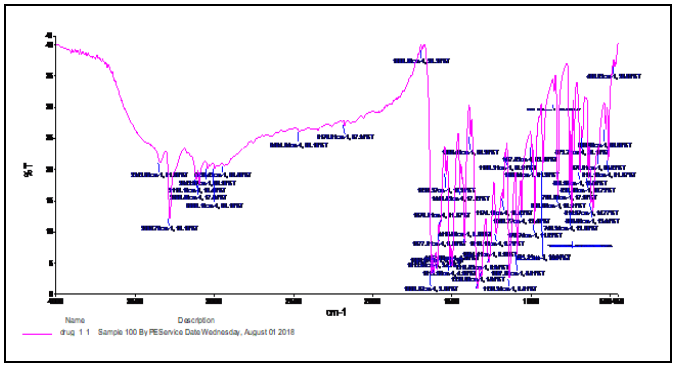

FIG. 6: FT-IR SPECTRA OF THE COPPER COMPLEX OF SCHIFF BASE LIGAND

TABLE 2: FTIR SPECTRAL DATA CONTAINING RELEVANT VIBRATIONAL BANDS OF THE LIGAND AND ITS COPPER (II) COMPLEX

S. no.	Compound	(C=O)	(C=N)	(C-S)	(Cu-N)
1	Schiff base $C_{52}O_8H_{44}N_{16}S_2$)	1615	1613	780.2	-
2	[Cu($C_{52}O_8H_{44}N_{16}S_2$)].$2H_2O$	1680	1590	780	600

H1 NMR Spectral Studies: In H NMR spectra of Cu (II) Complex in $CDCl_3$ solution was shown in **Fig. 7**. The following signals are exhibited by the Schiff base; phenolic -OH group at 11.59δ, phenyl as a multiplet at 7.94- 7.65δ, -N- CH_3 at 3.45, =C-CH_3 at 2.50δ. In the copper complex, all the peaks were slightly shifted to the downfield region due to metal- coordination [35-36].

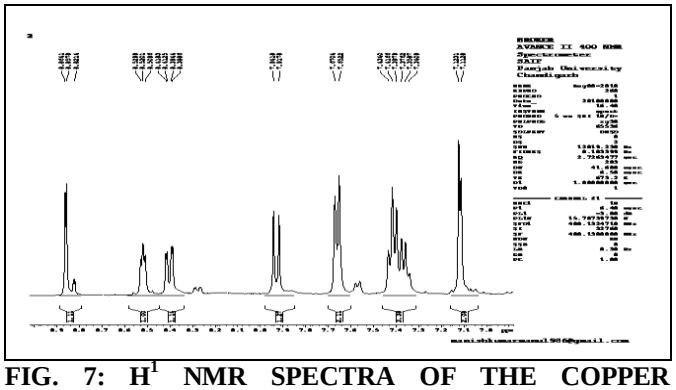

FIG. 7: H^1 NMR SPECTRA OF THE COPPER COMPLEX OF SCHIFF BASE LIGAND

XRD Studies: The X-Ray diffraction pattern of the Cu (II) complex is given in **Fig. 8**. A single crystal of the complex could not be isolated from any solvents. The powder XRD pattern of Cu (II) complex shows sharp crystalline peaks indicating its crystalline nature. All the peaks were observed in the XRD patterns of the copper complex and it also shows some additional peaks due to chelation. Highest intensity peak of the copper complex is observed at 26.528 °C. All the peaks are fairly sharpened in the complex which indicates that the Quantum confinement of Schiff Base by the copper ion [37]. The x-ray diffraction pattern of copper chelate insists reduced size in chelate than ligand owing to the increasing values of full width half maximum (FWHM). The crystalline sizes were calculated for prominent peaks for the prepared Schiff base copper complexes using Deye - Scherrer's formula [38].

$$D = 0.94\lambda/ß \cos \theta$$

Where λ is the wavelength of X-ray radiation, ß is the full width at half maximum of diffraction line and theta is the diffraction angle. Using the full width at half maximum intensity of the patterns, the average sizes of the particle is 26.5280.

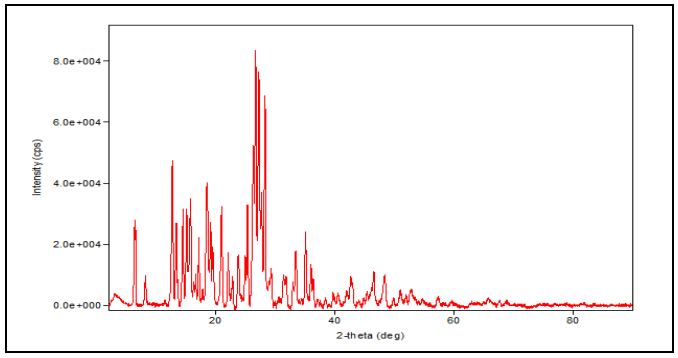

FIG. 8: POWDER XRD PATTERN OF THE COPPER COMPLEX OF SCHIFF BASE LIGAND [CU ($C_52O_8H_{44}N_{16}S_2$)].2H_2O

Thermo-Gravimetric Analysis: Thermo-gravimetric weight loss curves and the corresponding differential thermo-gravimetric (DTG) curves for the Schiff base ligand and its Cu (II) complex are shown in **Fig. 9** & **10**. The Schiff base ligand showed two well-defined steps at 133.69 - 326.26 (73.31%). The loss in weight its Cu (II) complex is 142 .18 - 313 .49 (10.034%). This large weight drop can be explained by considering that the residue as a 1:1 mixture of Cu_2O and CuO residues.

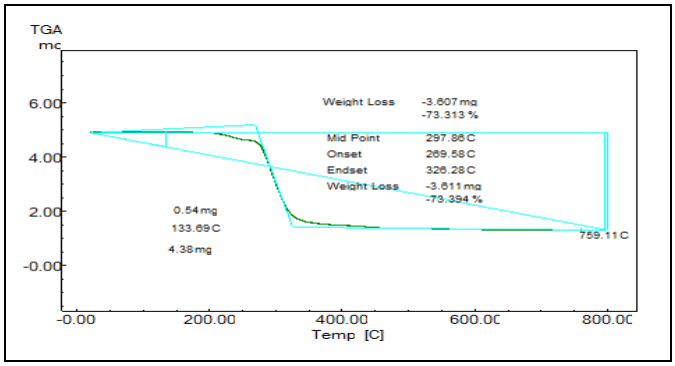

FIG. 9: TGA SPECTRA OF SCHIFF BASE LIGAND

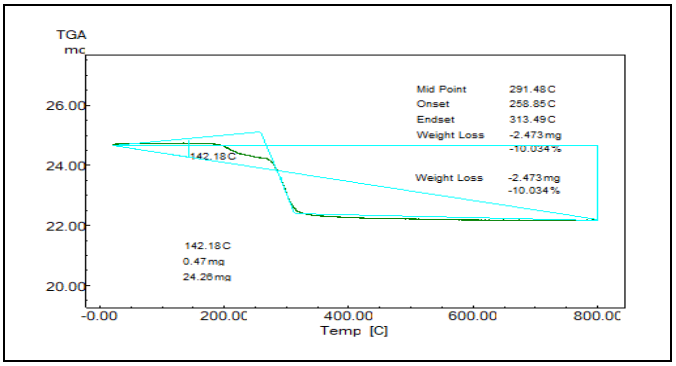

FIG. 10: TGA SPECTRA OF THE COPPER COMPLEX OF SCHIFF BASE LIGAND

TABLE 3: TGA DATA OF LIGAND AND ITS COPPER (II) COMPLEX

S. no.	Compounds	Temperature range (^oC)	Mass loss found (% calcd)	Assignment
1	[($C_{52}O_8H_{44}N_{16}S_2$)] Schiff base ligand	133.69 – 326.26	73.31	Loss of thiosemicarbazone group
2	[Cu($C_{52}O_8H_{44}N_{16}S_2$)].2H_2O	142 .18 – 313 .49	10.034	Formation of Cu_2O and CuO residues

Fluorescence Analysis: The emission of ligand and its Cu (II) complexes have been measured in the liquid state in room temperature. The fluorescence spectra of ligand and its Cu (II) complex are shown in **Fig. 11** & **12**. The ligand showed an intense emission band at 700 nm upon photo-excitation at 559 nm.

However, its Cu (II) complex exhibits a strong emission band at 475 nm and a weak band at 452 nm. The fluorescence intensity of the Schiff base ligand decreased drastically when coordinated with the Cu^{2+} metal ions. The decrease in the emission maxima was also observed in the Cu (II) complex when compared to its ligand.

TABLE 5: FLUORESCENCE DATA OF LIGAND AND ITS COPPER (II) COMPLEX

S. no.	Compound	Photo-Excitation	Emission band
1	$[(C_{52}O_8H_{44}N_{16}S_2)]$	559 nm	700 nm
2	$[Cu(C_{52}O_8H_{44}N_{16}S_2)].2H_2O$	452 nm	475 nm

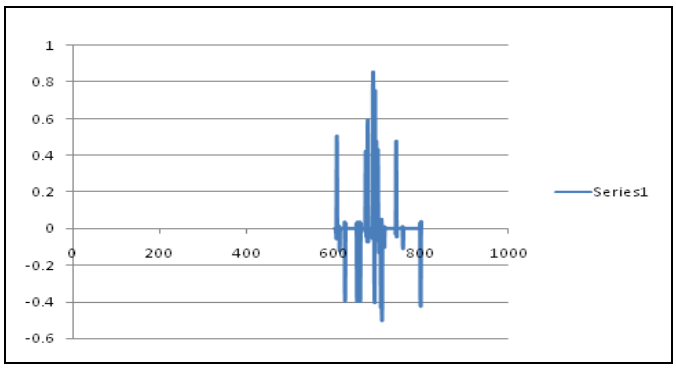

FIG. 11: FLUORESCENCE SPECTRA OF SCHIFF BASE LIGAND

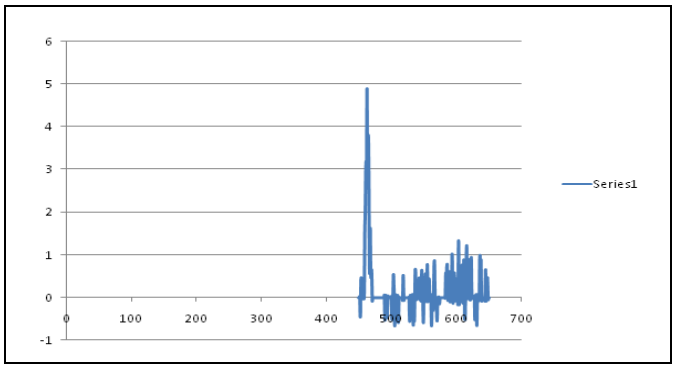

FIG. 12: FLUORESCENCE SPECTRA OF THE COPPER COMPLEX OF SCHIFF BASE LIGAND

Molecular Docking Analysis: In this study the crystal structure of topoisomerase IIα (PDB: 4fm9), was used for identification of binding modes of Schiff base ligand and copper (II) complex through molecular docking study, is composed of three subunits *i.e.* A, C, and D therefore only A subunit was used for the docking analysis. The copper complex could not afford any hydrogen bond with the active site of target enzyme but have a significant binding affinity (-19.8 kcal/mol) compared to Schiff base ligand which has -8.9 kcal/mol. These results reveal here that significant binding affinity of the copper complex might be responsible for the anti-cancer activity in the humans. Different amino acids interactions obtained through molecular docking study also suggest that copper complex 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.

TABLE 5: DOCKING STUDIES OF LIGAND AND ITS CU (II) COMPLEX

S. no.	Ligand receptor	Binding affinity (kcal/mol)	Amino acids involved in interactions	No. of H-bond
1.	Schiff base $C_{52}O_8H_{44}N_{16}S_2)$	-8.9	Gln544, Lys614, Glu712, Arg713, Pro724, Gly725, Lys729, His758	NIL
2.	$[Cu(C_{52}O_8H_{44}N_{16}S_2)].2H_2O$	-19.8	Lys489,His548,Lue552, Phe653, Lys655, Arg661,Pro724,Gly725,Gln726,Tyr757, His759,Asn779,Ile856,Thr858, Gly859, Arg929	NIL

DPPH Activity: In DPPH free radical scavenging activity antioxidants are reacting with the stable free radical 1, 1 diphenyl picryl hydrazyl (DPPH) producing a colorless 1, 1 diphenyl-2-picryl-hydrazine. When DPPH receives an electron or hydrogen radical to become more stable, its absorption decreases [39]. The DPPH scavenging activity was expressed as IC_{50}, whose concentration is sufficient to obtain 50% of maximum scavenging activity. The IC_{50} values of ligand and its Cu (II) complex are depicted in **Table 6**. Ascorbic acid was used as a standard. The result illustrates the influence of Cu (II) complex on the initiation of DPPH antioxidant activity. This result clearly indicates that the copper complex shows higher antioxidant activities. The free radical scavenging activity of the compounds depends on the structural features [40].

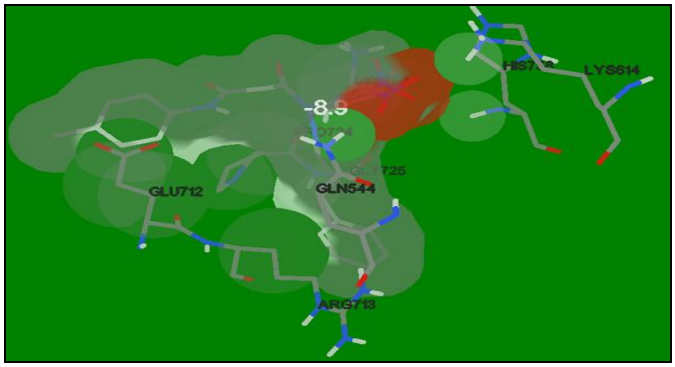

FIG. 13: A BINDING MODE OF SCHIFF BASE LIGAND WITH TOPOISOMERASE IIα (3D MODEL OF INTERACTIONS BETWEEN LIGAND AND TARGET)

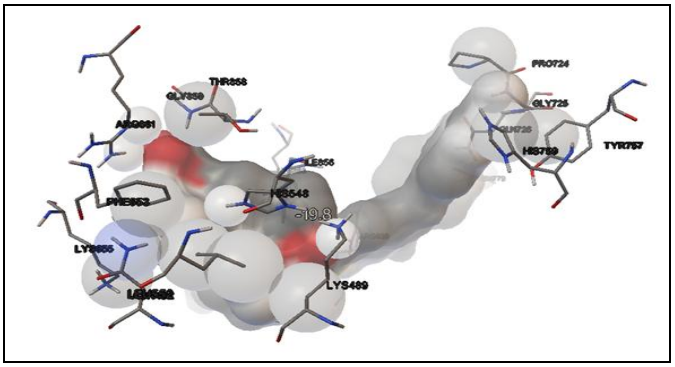

FIG. 14: A BINDING MODE OF THE COPPER COMPLEX WITH TOPOISOMERASE IIα (3D MODEL OF INTERACTIONS BETWEEN COPPER COMPLEX AND TARGET)

DPPH Activity: In DPPH free radical scavenging activity antioxidants are reacting with the stable free radical 1, 1 diphenyl picryl hydrazyl (DPPH) producing a colorless 1, 1 diphenyl-2-picryl-hydrazine. When DPPH receives an electron or hydrogen radical to become more stable, its absorption decreases [39]. The DPPH scavenging activity was expressed as IC_{50}, whose concentration is sufficient to obtain 50% of maximum scavenging activity. The IC_{50} values of ligand and its Cu (II) complex are depicted in **Table 6**. Ascorbic acid was used as a standard. The result illustrates the influence of Cu (II) complex on the initiation of DPPH antioxidant activity. This result clearly indicates that the copper complex shows higher anti-oxidant activities. The free radical scavenging activity of the compounds depends on the structural features [40].

TABLE 6: IC_{50} VALUES OF DPPH RADICAL SCAVENGING ACTIVITY OF LIGAND AND ITS CU (II) COMPLEX

S. no.	COMPOUND	IC_{50} µg/ml
1	Schiff base $C_{52}O_8H_{44}N_{16}S_2$)	122.42 ± 2.76
2	[Cu($C_{52}O_8H_{44}N_{16}S_2$)].$2H_2O$	130.52 ± 3.76

The result showed that the scavenging activity of ligand was increased when coordinating with a copper ion.

Anti-cancer Activity: The anti-cancer activity of ligand and its copper (II) complex was determined by MTT assay. The IC_{50} values of ligand and Cu (II) complex are depicted in **Table 7**. The pharmacological testing has proved that the cytotoxic effect of Cu (II) complex was considered moderate to less pronounced compared to the standard drug Cisplatin. However, the copper complex has a higher inhibitory effect than its ligand. Several compounds in particular copper complexes of Schiff base ligand endowed with significant cytotoxic potency and can be considered as new lead compounds for further modifications.

TABLE 7: IC_{50} VALUES OF ANTICANCER ACTIVITY OF LIGAND AND CU (II) COMPLEX

S. no.	Compounds	IC_{50}(µg/ml)
1	Schiff base $C_{52}O_8H_{44}N_{16}S_2$)	54.69 ± 0.2
2	[Cu($C_{52}O_8H_{44}N_{16}S_2$)].$2H_2O$	34.79 ± 0.3
3	Cisplatin standard	21.49 ± 0.4

CONCLUSION: A novel Schiff base ligand and its Cu (II) complex have been prepared and characterized by various spectroscopic techniques. Copper complex showed good cytotoxic activity against MCF-7 cancer cell line. The docking results indicate that copper complex was found to be fitted into the active site of the enzyme and have maximum binding energy (-19.4 kcal/ mol) and demonstrated the highest affinity towards target enzyme along with occupying the quite similar active site as Schiff base ligand occupied. Cu (II) complex showed considerable anticancer activity against MCF-7 cell lines and more pronounced antioxidant activity in the presence of DPPH. The cytotoxicity screening of some of the new compounds revealed that the selected compounds showed reasonable antitumor activity against MCF-7 cancer cell line in comparison to the traditional anticancer drugs such as Cisplatin.

ACKNOWLEDGEMENT: The authors are thankful to the management of ITM University for providing the lab facilities and the Director, SAIF, Chandigarh for recording NMR spectra. The authors also acknowledge the Radiant research

laboratory, Bangalore for providing the antioxidant data and anticancer activity studies.

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

REFERENCES:

1. Iakovidou Z, Papageorgiou A, Demertzis MA, Mioglou E, Mourelatos D, Kotsis A, Yadav PN and Kovala-Demertzi D: Platinum (II) and palladium (II) complexes with 2-acetylpyridine thiosemicarbazone: cytogenetic and anti-neoplastic effects 2001; 12(1): 65-70.
2. Patole J, Dutta S, Padhye S and Sinn E: Tuning up superoxide dismutase activity of copper complex of salicylaldehyde semicarbazone by heterocyclic bases pyridine and N-methyl imidazole. Inorg Chim Acta 2001; 318: 207-11.
3. Maure RI, Blower PI, Dilworth IR, Reynolds CA, Zheng Y and Mulleu GED: Studies on the mechanism of hypoxic selectivity in copper bis (Thiosemicarbazone) radio-pharmaceuticals. J Med Chem 2002; 45(7): 1420-31.
4. Cowly AR, Dilworth IR, Donnely PS, Labisbdl E and Sousa A: An unusual dimericstructure of a Cu(I) bis (thiosemicarbazone) complex: implications for the mechanism of hypoxic selectivity of the Cu(II) Derivatives. J Am Chem Soc 2002; 124(19): 5270-71.
5. Ferrari M B, Bisceglie F, Pelosi G, Sassi M, Tarasconi P, Cornia M, Capacchi S, Albetini R and Pinelli S: Synthesis, characterization and X-ray structures of new anti-proliferative and proapoptotic natural aldehyde thiosemicarbazones and their nickel (II) and copper (II) complexes. Journal of Inorganic Biochemistry 2002; 90(3-4): 113-26.
6. Jouad el M, Thanh XD, Bouet G, Bonneau S and Khan MA: *In-vitro* and *in-vivo* effects of [Ni (M5FTSC)2Cl_2] complex in cancer: preliminary tests. Anti-cancer Res 2002; 22(3): 1713-6.
7. Gray HB: Biological inorganic chemistry at the beginning of the 21st century. Proc Natl Acad Sci USA. 2003; 100(7): 3563-8.
8. Abrams MI and Murrer BA: Metal compounds in therapy and diagnosis. Science 1993; 261(5122): 725-30.
9. Funston AM, Ghiggino KP, Gannas MJ, Mc. Fadyen WD and Tregoan PA: Synthesis, structural studies and photochemistry of Co (II) complexes of anthracenyl cyclam macrocycles. J Chen Soc Dalton Trans 2003; 19: 3704-12.
10. Sorenson JRJ: Copper-complexes in biochemistry and pharmacology. Chem Br 1984; 16: 1110.
11. Gouch RK, Kensler TW, Oberlew LW and Sorenson JRJ: Biochemical and Inorganic Copper Chemistry, (Adenine, New York) 1986; 1: 139.
12. Cvek B, Milacic V, Taraba J and Dou QP: Ni (II), Cu (II), and Zn (II) diethyldithiocarbamate complexes show various activities against the proteasome in breast cancer cells. J Med Chem 2008; 51(20): 6256-8.
13. Anderson RF, Denny WA, Ware DC and Wilson WR: Pulse radiolysis studies on the mechanism of hypoxia-selective cytotoxicity of a cobalt-mustard complex. Br J Cancer 1996; 74(Suppl. 27): S48-S51.
14. Teicher BA and Holden SA: Anti-tumour and radio-sensitizing activity of several platinum-(+) dye complexes. Radiat Res 1987; 109(1): 58-67.
15. Teicher BA, Abrams MJ, Rosbe KW and Herman TS: Cytotoxicity, Radiosensitization, Anti-tumor Activity, and Interaction with Hyperthermia of a Co (III) mustard complex. Cancer Res 1990; 50(21): 6971-75.
16. Ware DC, Siim BG, Robinson KG, Denny WA, Brothers PJ and Clark G R: Synthesis and characterization of aziridine complexes of cobalt (III) and chromium (III) designed as hypoxia-selective cytotoxins. X-ray crystal structure of trans-[Co(Az)4(NO_2)2]Br.cntdot.2H_2O. cntdot. Li Br. Inorg Chem 1991; 30 (19): 3750-57.
17. Ware DC, Palmer BD, Wilson WR and Denny WA: Hypoxia-selective antitumor agents. 7. Metal complexes of aliphatic mustards as a new class of hypoxia-selective cytotoxins. Synthesis and evaluation of cobalt (III) complexes of bidentate mustards. J Med Chem 1993; 36(13): pp 1839-46.
18. Wilson WR, Moselen JW, Cliffe S, Denny WA and Ware DC: Exploiting tumor hypoxia through bioreductive release of diffusible cytotoxins: the cobalt(III)-nitrogen mustard complex SN 24771. Int J Radiat Oncol Biol Phys 1994; 29(2): 323-7.
19. Ware DC, Palmer HR, Brothers PJ, Rickard CEF, Wilson WR and Denny WA: Bis-tropolonato derivatives of Cobalt (III) complexes of bidentate aliphatic nitrogen mustards as potential hypoxia-selective cytotoxins. Journal of Inorganic Biochemistry 1997; 68(3): 215-24.
20. Blower PJ, Dilworth JR, Maurer RI, Mullen GD, Reynolds CA and Zheng Y: Towards new transition metal-based hypoxic selective agents for therapy and imaging. Journal of Inorganic Biochemistry 2001; 85(1): 15-22.
21. Singh DP, Kumar R and Singh J: Synthesis and spectroscopic studies of biologically active compounds derived from oxalyldihydrazide and benzil, and their Cr (III), Fe(III) and Mn (III) complexes. European Journal of Medicinal Chemistry 2008, 44(4): 1731-36.
22. Fraústo da Silva JJR and Williams RJP: The Biological Chemistry of the Elements. Oxford University Press, Oxford, UK 1991.
23. Parkin G: Synthetic analogues relevant to the structure and function of zinc enzymes. Che Rev 2004; 104(2): 699-68.
24. Cleare MJ, Hughes EG, Jacoby B and Pepys J: Immediate (type I) allergic responses to platinum compounds. Clin Allergy 1976; 6(2): 183-95.
25. Singh AP, Kaushik NK, Verma AK, Hundal G and Gupta R: Synthesis, structure and biological activity of copper(II) complexes of 4-(2-pyridylmethyl)-1, 7-dimethyl-1, 4, 7-triazonane-2, 6-dione and 4-(2-pyridylethyl)-1,7-dimethyl-1,4,7-triazonane-2, 6-dione. Eur J Med Chem 2009; 44(4): 1607-14.
26. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ: Cancer statistics 2008. CA Cancer J Clin 2008; 58: 71-96.
27. Hu WP, Yu HS, Chen YR, Tsai YM, Chen YK, Liao CC, Chang LS and Wang JJ: Synthesis and biological evaluation of thioben- zanilides as anticancer agents. Bio Org Med Chem 2008; 16: 5295-02.
28. Hussain S, Slevin M, Ahmed N, West D, Choudhary MI, Naz H and Gaffney J: Stilbene glycosides are natural product inhibitors ofFGF-2-induced angiogenesis. BMC Cell Biol 2009; 10: 30-31.
29. Kemnitzer W, Jiang S, Wang Y, Kasibhatla S, Crogan GC, Bubenik M, Labrecque D, Denis R, Lamothe S, Attardo G, Gourdeau H, Tseng B, Drewe J and Cai SX: Discovery of 4-aryl-4H-chromenes as a new series of apoptosis inducers using a cell- andcaspase-based HTS assay. Part 5: modifications of the 2- and 3-positions. Bio Org Med Chem Lett 2008; 18: 603.
30. Lee ER, Kang YJ, Choi HY, Kang GH, Kim JH, Kim BW, Han YS, Nah SY, Paik HD, Park YS and Cho SG:

Induction of apoptotic cell death by synthetic naringenin derivatives in human lung epithelial carcinoma A549 cells. Biol Pharm Bull 2007; 30: 2394-98.
31. Burden DA and Oshereroff N: Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme. Biochim Biophys Acta 1998; 1400: 139-54.
32. Wendorff TJ, Schmidt BH, Heslop P, Austin CA and Berger JM: The structure of DNA-Bound human topoisomerase II alpha: conformational mechanisms for coordinating inter-subunit interactions with DNA cleavage. J Mol Biol 2012; 424; 109-24.
33. Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS and Olson AJ: Computational protein–ligand docking and virtual drug screening with the Auto Dock suite. Nature Protocols 2016; 11(5): 909-19.
34. Braca A, Tommasi, ND, Bari LD, Pizza C, Politi M and Morelli I: Anti-oxidant principles from *Bauhinia terapotensis*. J Nat Prod 2001; 64: 892-95.
35. Saha MR, Hasan SMR, Aktar R. Hossain MM, Alamb MS, Alam MA and Mazumdar MEH: *In-vitro* free radical scavenging activity of methanol extract of the leaves of *Mimosops elengi* Linn. Bangl. J Vet Met 2008; 6: 197-02.
36. Sargent TM and Taylor CG: Appraisal of the MTT as a rapid test of chemosensitivity in acute myeloid leukemia. Br J Cancer 1989; 60: 206-10.
37. Dziewulska-Kuaczkowska, A: Manganese(II), cobalt(II), nick- el (II), copper (II) and zinc (II) complexes with 4-oxo-4H-1-benzo- pyran-3-carboxaldehyde. Therm Anal Calorim 2010; 101: 1019-26.
38. Rosu T, Pahontu E, Maxim C, Georgescu R, Stanica N, Almajan GL and Gulea A: Synthesis, characterization and antibacterial activity of some new complexes of Cu(II), Ni (II), VO (II), Mn (II) with Schiff base derived from 4-amino-2, 3- dimethyl-1-phenyl-3-pyrazolin-5-one. Polyhedron 2010; 29: 757-66.
39. Soliman AA and Mohamed GG: Study of the ternary complexes of copper with salicylidene-2-aminothiophenol and some amino acids in the solid state. Thermochim Acta 2004; 421: 151-9.

How to cite this article:
Rai S, Agrawal A and Kothari R: Synthesis, identification of *in-silico* interactions and biological studies of novel schiff base ligand and its copper (II) complex. Int J Pharm Sci & Res 2020; 11(2): 745-54. doi: 10.13040/IJPSR.0975-8232.11(2).745-54.