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SYNTHESIS, CHARACTERIZATION OF NOVEL COPPER (II) COMPLEXES OF SCHIFF BASE MOLECULAR ADDUCTS OF DOXORUBICIN AND THEIR CYTOTOXIC ACTIVITY AGAINST MCF-7 CELL LINES

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ABSTRACT

In the present study a series of Cu(II) complexes of Schiff base molecular adducts of doxorubicin have been synthesized and characterized by analytical, molar conductance, IR electronic magnetic susceptibility and powder XRD measurements and screened for various biological activities (antioxidants, and cytotoxicity). In all the Cu(II) complexes 1:2 metal to ligand molar ratio was obtained from analytical data. The molar conductance data confirm that all complexes are non- electrolytic in nature. Based on the electronic and magnetic data, an distorted octahedral geometry is ascribed for all the copper(II) complexes. All Cu (II) complexes showed considerable anticancer activity against MCF-7 cell lines and more pronounced antioxidant activity lines and more pronounced antioxidant activity in the presence of DPPH. The newly synthesized title compounds were evaluated for their MCF-7 cell growth inhibition, the results revealed that all the tested compounds posseses inhibitory effects on the growth of MCF-7 cancer cells. Compound 3 showed the highest inhibition activity against MCF-7 cell line (IC_{50} 7.21 ± 0.1 µg/ml) among all tested Compounds.

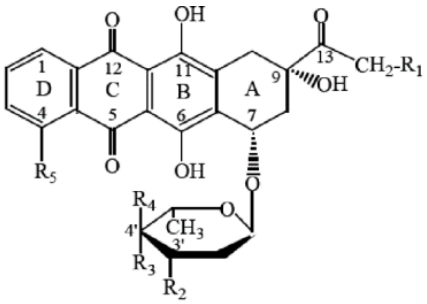
KEYWORDS: Copper (II) complexes, Anticancer, Antioxidant, Cytotoxic activity, MCF -7 cell line.

1. INTRODUCTION

Metal-based antitumor drugs play a relevant role in antiblastic chemotherapy. Cisplatin is regarded as one of the most effective drugs, even if severe toxicities and drug resistance

phenomena limit its clinical use. Therefore, in recent years there has been a rapid expansion in research and development of novel metal-based anticancer drugs to improve clinical effectiveness, to reduce general toxicity and to broaden the spectrum of activity. Doxorubicin (also called adriamycin) belongs to a class of compounds with similar structures, called anthracyclines. Like daunorubicin, the first anthracycline compound to be described, doxorubicin was isolated from Streptomyces peucetius, a soil bacterium.[1,2] Doxorubicin has shown great efficacy in cancer cell killing for both solid and liquid tumors, but the emergence of drug resistance and potential side effects such as heart muscle damage after doxorubicin treatment are major limitations for successful cancer treatment.[3] A number of models have been proposed for doxorubicin-mediated cell death, including topoisomerase II poisoning, DNA adduct formation, oxidative stress, and ceramide overproduction.[4–6] Anthracycline drugs such as doxorubicin are mostly planar molecules that preferentially intercalate between neighboring DNA base pairs, anchored on one side by one or more sugar moieties that sit in the DNA minor groove. When DNA is topologically constrained, as in the case of plasmid circles, the strand separation that occurs during intercalation unwinds the double helix and produces DNA supercoils, resulting in increased torsional stress. Linear genomes of eukaryotes are partitioned into independent topological domains by protein factors such as insulator binding protein CTCF[7], so each domain is topological constrained. In vitro studies suggest that torsional stress can affect the structure and dynamics of nucleosomes, the repeating unit of chromatin composed of DNA wrapped around octameric histone cores.[8,9] Interestingly, recent in vivo studies implicate doxorubicin in nucleosome eviction and replacement.[10,11] Taken together, torsion-induced nucleosome destabilization is emerging as a significant molecular mechanism for the action of doxorubicin and related anthracycline drugs. The most commonly diagnosed cancers worldwide are lung cancer, breast cancer and colorectal cancer, however, in women, breast cancer is the most common malignant tumor. This is the most frequently occurring cancer among women especially in developed countries. Breast cancer is characterized by following features: frequent aggressive invasion, early metastasis and resistance to multiple drugs used in therapy. All the above mentioned factors suggest the need for the exploration of the novel, more efficient anticancer agents without disruptive side effects. Doxorubicin (DOX) belongs to the anthracycline group—a class of drugs that are commonly used for breast cancer chemotherapy, often in conjunction with other compounds.[12] In the chemical structure of anthracyclines two parts can be distinguished: the aglycone, that consists of four rings two of which are aromatic rings (B and D), and the saccharide moiety (Figure1). Rings indicated by symbols A, B, C and D differ

from each other. Ring B with the hydroquinone structure differs from the C ring, which is devoid of hydroxyl groups. The side chain is located at the C-9 position of the A ring. It is linked to the carbonyl group and at the C-7 position of the amino sugar (daunosamine) and it is attached by a glycosidic linkage. Ring D has in turn a methoxyl group in the C-4 position. In the anthracycline antibiotic molecule several asymmetric carbon atoms can be distinguished: two of them in the aglycone (C-7 and C-9) and four in the sugar moiety (C-1, C-3, C-4, C-5). The presence of a carboxyl group (ring A), hydroxyl group (ring B) and the sugar moiety affect the interactions of the macromolecules within cells.[13]



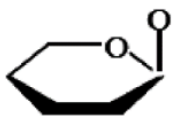
	R_1	R_2	R_3	R_4	R_5
daunorubicin	H	NH_2	OH	H	OCH_3
idarubicin	H	NH_2	OH	H	H
doxorubicin	OH	NH_2	OH	H	OCH_3
epidoxorubucin	OH	NH_2	H	OH	OCH_3
pirarubicin	OH	NH_2		H	OCH_3

Figure 1: Structural formula of the selected anthracyclines regarding the functional groups of the individual compounds.

The chemical structure of doxorubicin is characteristic for the anthracycline antibiotics belonging to the class I, such as DOX, daunorubicin, epirubicin, idarubicin, pirarubicin.[14] Anthracyclines contain in their chemical structure a fragment of both hydrophilic and hydrophobic properties, which allows them to binding to the plasma proteins and cellular membranes. Due to their polar nature they dissolve in water and display both acidic and basic properties. These compounds have crystalline structures and are stable at room temperature.[15] They are known to have cytotoxic properties against tumor cells through three different mechanisms. The first one is the intercalation between strands of DNA/RNA molecules, which results in interference with DNA/RNA synthesis in rapidly dividing cells, such as breast cancer cells.[16] The second mechanism is the inhibition of topoisomerase II activity, which is based on the binding of the anthracycline with the

DNA-topoisomerase complex. Anthracyclines, such as doxorubicin, belong to the group of topoisomerase II poisons and therefore they may immobilize topoisomerase II-DNA complexes. For this reason they cause an inhibition of the release of DNA breaks generated by the enzyme. According to the literature, "catalytic inhibitors" inhibit enzymes binding to DNA substrate or cause the closing of the topoisomerase II in the form of a bracelet that surrounds DNA at the post-religation step. However, only topoisomerase II poisons activity can produce DNA breaks.[17] The third mechanism by which selected compounds from the anthracycline group influence cancer cells metabolism is the creation of iron-mediated oxygen free radicals.[18]

Literature data indicate that the pharmacological properties of anthracyclines are indirectly connected with the presence of metal ions and with the environment of the metal-antibiotic chelates.[19,20] The investigations associated with metals such as Cu(II), Hg(II), Ag(I), Ni(II) and Mg(II) binding to anthracyclines have shown that the metal has the possibility to bound both to aglycone ring and to the sugar moiety—daunosamine. Furthermore, a considerable limitation is the solubility of the drug. Close to the solubility ratio metal-anthracyclines complexes have the capacity to create dimers.[21] The complexation of the anthracyclines by the metal ions led to discovery of the new, less toxic anticancer agents. The structure of the complexes, the impact that they have on redox reactions and the characteristics of the selected metal binding is still under investigation.[22-24] The locations where the metal is bound to the anthracycline are aglycone inner rings depending on the metal nature, with the simultaneous consideration of the ligand spatial structure.

Metal -DOX complexes, by the increase of the oxidative stress level, could potentially induce apoptosis. Fe^{3+}-DOX complex bound up with DNA, is stable in aqueous solution and in addition it can be reduced to Fe^{2+} through the action of reducing agents such as NADPH dependent cytochrome P_{450} reductase, glutathione or cysteine. These reactions are accompanied by the creation of superoxide anion and the conversion of anthracycline quinone to semiquinone free radicals. During the Haber-Weiss reaction, which is catalyzed by iron ions, hydrogen peroxide and highly reactive hydroxyl radicals are produced. Semiquinone radicals have the ability to transform into a C7 aglycone radical, which is a potent alkylating agent. Reactive oxygen species created with the participation of anthracyclines, cause DNA damage and lead to the apoptosis.[25,26] Apoptosis is a kind of programmed cell death, that occurs in multicellular organisms and it is important for homeostasis, normal cells and tissues growth

and development, but also for cancer treatment. Any changes in the process of apoptosis significantly influence cells and tissues metabolism, e.g., may cause an abnormal cell growth, uncontrolled cell divisions and generation of mutations. Consequently, the control and regulation of apoptosis is a main target for new anticancer therapies.[27,28] In mammalian cells exist at least two pathways, by which an apoptosis occurs: an extrinsic death-receptor dependent apoptosis and intrinsic mitochondrial dependent apoptosis. In both above mentioned pathways the induction of cell death is connected with selected caspases activation: initiator caspases (e.g., caspase-8 and -9) and effector caspases (e.g., caspase-3, -6, and -7). The purpose of present study was to evaluate the influence of selected, newly synthesized copper – Schiff base molecular adducts of doxorubicin complexes on breast cancer cells proliferation, viability, cytotoxicity and apoptosis. The molar ratio of created copper - Schiff base molecular adducts doxorubicin DOX complexes was determined by spectrophotometric titration in buffer solution at pH = 7.00. FT-IR spectra of doxorubicin and Schiff base molecular adducts of doxorubicin copper complexes and UV-Vis spectra of complexes were taken. Because DOX and other anthracyclines cause severe side effects, a need to synthesize new, more efficient DOX complexes with copper metals arises.

The present work is a part of the project involving several different aspects. The first one is the evaluation of antitumor activity of copper complexes as compared to Schiff base molecular adducts of doxorubicin drug. The second are relational studies regarding molecular structure and distribution of electrical charge and cytotoxic effects of studied compounds. The third aspect is the study of synergistic effects, in mutual reinforcement of antineoplastic action of metal and ligand (with proven cytotoxic activity).

2. EXPERIMENTAL

2.1. Materials

The reagents and chemicals were obtained from the commercial sources and used as received. Solvents were purified as reported earlier. DOX hydrochloride was obtained from Sigma-Aldrich. Copper sulphate, Methanol, Ethanol, petroleum Ether and Tris-HCl buffer were obtained from Merck company and solvents used were of AR grade.

2.2. Physical measurements

All 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 ligands and their Cu(II) complexes were recorded on Perkin Elmer FTIR spectrophotometer within the range of 4000- 400 cm^{-1} using KBr disc, Molar conductance of the complexes was measured using a Digisun conductivity meter in DMF. The electronic absorption spectra of the ligands and their Cu (II)complexes were recorded using perkin Elmer Spectrophotometer from 200 -800nm.. The X rays pattern of the complexes were recorded on Xpert- ProX ray diffratrometer with CuK radiation (^=1.546A^0). All reactions were monitored by TLC using pre-coated Aluminum sheet silica gel Merck 60 F 254 and were visualized by UV lamp. Chemical naming, calculation of molecular weight (M.wt.) of new compounds were performed by ChemBio- Draw 12 software.

2.3 Synthesis of of Schiff base molecular adducts of Doxorubicin drug

Doxorubicin (0.02 mM) was weighed and dissolved in 10 mL water. NaOH was added to the solution to provide a slightly alkaline pH. Thereafter, the stoichiometric amount of alcoholic solution of aromatic aldehyde/ketones was continuously added under stirring for one hour. The reaction mixture was then stirred for 48 h at room temperature. The obtained solution was incubated for two days to precipitate the sediment compounds. After precipitation, the compounds were filtered with water and dried under vacuum. Elemental composition of the compounds was determined. The obtained complexes had [Cu (DOX. $L_{1\text{-}4}$]$(SO_4)_2$ composition.

2.4 Template Synthesis of Copper complexes of *Schiff base molecular adducts of Doxorubicin drug*[1-4]

All copper complexes were synthesized according to the method published previously. Briefly, a mixture of the appropriate hydrated copper salt in absolute ethanol and Schiff base molecular adducts of doxorubicin in absolute ethanol were added slowly with stirring. After the addition of all reagents, the reaction was carried out for 8 hrs under reflux. The solvent was evaporated under reduced pressure and the residue obtained was quenched with ethanol. Precipitate was filtered off, washed with ether and dried in vacuum.

2.5 BIOLOGICAL STUDIES

2.5.1 DPPH radical scavenging activity

The free radical scavenging activity of the Cu(II) complexes were determined by using DPPH free radical scavenging method according to the literature. Compounds were dissolved in DMSO (1mg/ml) and used as stock solutions. From the stock solutions, 0.01ml of each

compound solution with different concentration(2500- 7.8 µg/ml for SL1, SL3, SL4 compounds) and (1000-62.5µg/ml) for SL2 compound were added to 0.2 ml of methanolic solution of DPPH. All the tubes were incubated at 37ºC for 30 minutes. After incubation 0.1ml of reaction mixture was pipette out to microlitre plate. Aborbance was measured at 490nm using microlitre reader. Same procedure was reapeated for standard by replacing test samples with standard test and control was performed in triplicate and test blank and control blank were conducted in singlet. The percentage of scavenging activity of DPPH free radical was measured by using the following formula,

$$\text{Scavenging activity}(\%) = \frac{Ao - Ai}{A0}$$

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

2.5.2 Anticancer activity

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

MTT ASSAY

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

$$\text{Cytotoxicity} = 1 - \frac{O.D\ of\ treated\ cells}{O.D\ of\ untreated\ cells}$$

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

3. RESULTS AND DISCUSSION

The physical and analytical data of (II) complexes are depicted in Table 1. All copper complexes are colored and very stable at room temperature, soluble in DMF and DMSO. Analytical data confirm the metal to ligand ratio is 1 : 2 in all the copper complexes. The molar conductance measurements of the complexes were recorded in DMF ($1x10^{-3}$). The results indicate their non-electrolytic nature

Table 1: The physical and analytical data Cu (II) complexes of Schiff base molecular adducts of doxorubicin.

S.No.	Compound	Molecular weight(g/mol)	Colour	Yield%	Elemental analysis					Molecular conductivitymho^{-1} mol^{-1} cm$^{2}\lambda$
					C	H	N	S	Cu	
1.	[Cu $C_{70}H_{42}N_2O_{19})_2](SO_4)_2$]	194	Maroon	79.2%	64.1 (64.38)	3.5 3(3.49)	4.49 (4.63)	10.28 (10.30)	0.12 (9.63)	34.1
2.	[Cu ($C_{70}H_{42}N_2O_{19}Cl)_2](SO_4)_2$]	196	Muddy brown	72.3%	62.49 (61.23)	3.48 (3.20)	4.33 (4.21)	0.16 (10.19	.45 9.52)	29.3
3.	[Cu ($C_{68}H_{40}N_2O_{21})_2](SO_4)_2$]	1166	Dark orange	65.9%	60.45 (59.3)	.24 (3.45)	4.45 (4.26)	10.22 (10.16)	10.92 (10.50)	19
4.	[Cu ($C_{70}H_{44}N_2O_{19})_2](SO_4)_2$]	1267	Dark red	59.57%	61.63 (61.68)	3.16 (3.21)	4.35 (4.48)	10.20 (10.21)	10.22 (10.18)	19.3

3.1 FTIR spectral studies

The FTIR spectral data containing relevant vibrational bands of the ligands and their Cu(II) complexes are listed in table 2. The ligands showed a band in the range of 1615-1680 cm^{-1} which is due to υ (c=0) group of the Schiff base moiety, this band was shifted to lower wave number region 5-75cm^{-1} in their corresponding Cu(II) complexes, indicating the coordination of nitrogen atom of the Schiff base ligand.[29] The stretching vibration of the azomethine group (C=N) was observed in the range of 1613-1590 cm^{-1} in all the ligand. This band was shifted to 30-40 cm^{-1} lower wavenumber region in their Cu(II) complexes, indicating the participation of nitrogen atom of azomethine group in coordination to the metal ion.[30] Further the coordination of nitrogen was supported by the appearance of a non-ligand bands at 600-400 cm^{-1} region due to the (Cu-N), respectively. From the above spectral data it was concluded that Schiff Base ligands acts as monobasic tridentate ligands with NNS donor sites. The FTIR spectral data containing relevant vibrational bands of the ligands and their copper(II) complexes are listed in table 2.

Table 2: The FTIR spectral data containing relevant vibrational bands of the ligands and their copper(II) complexes.

S.No.	Compound	U (C=N)	U (C-S)	U (M-N)
1.	$[Cu\ C_{70}H_{42}N_2O_{19})_2](SO_4)_2]$	151	756	534
2.	$[Cu\ (C_{70}H_{42}N_2O_{19}Cl)_2](SO_4)_2]$	1570	758	530
3.	$[Cu\ (C_{68}H_{40}N_2O_{21})_2](SO_4)_2]$	1572	750	520
4.	$[Cu\ (C_{70}H_{44}N_2O_{19})_2](SO_4)_2]$	1590	754	536

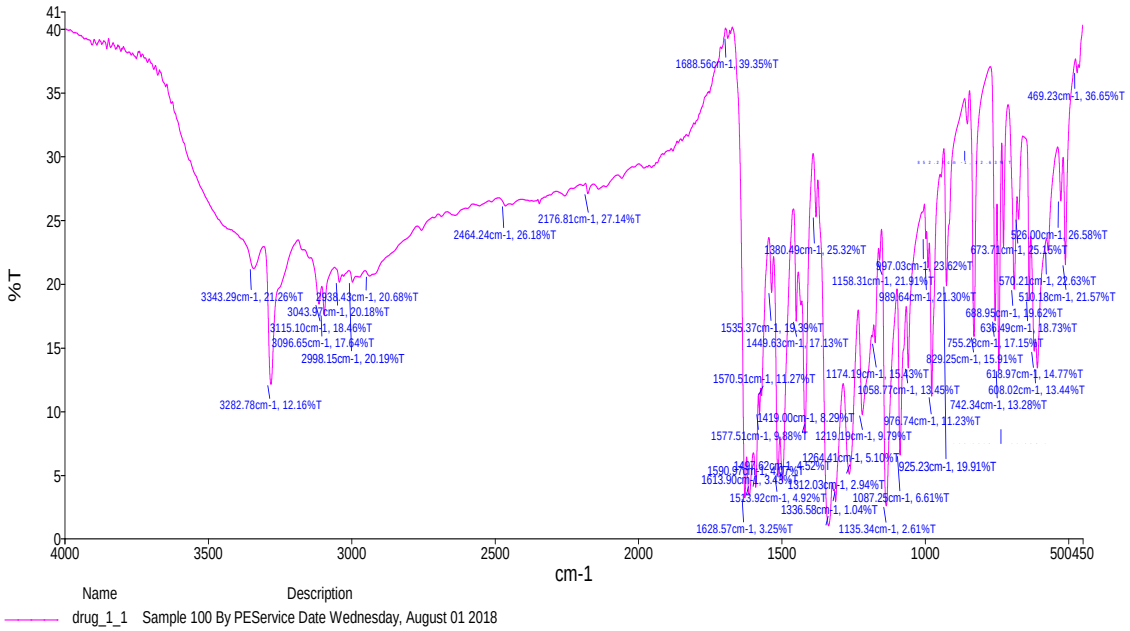


Figure 1: FTIR spectra of $[Cu\ C_{70}H_{42}N_2O_{19})_2](SO_4)_2]$ complex.

3.2. H NMR Spectral studies

In H NMR spectra of Cu(II) Complexes in $CDCl_3$ solution was shown in figure 2. The following signals are exhibited by the Schiff base; phenolic -OH group at 11.59 δ, phenyl as multiplet at 7.94- 7.65δ, -N-CH_3 at 3.45, =C-CH_3 at 2.50δ. In copper complexes, all the peaks were slightly shifted to downfield region due to metal- coordination.[31]

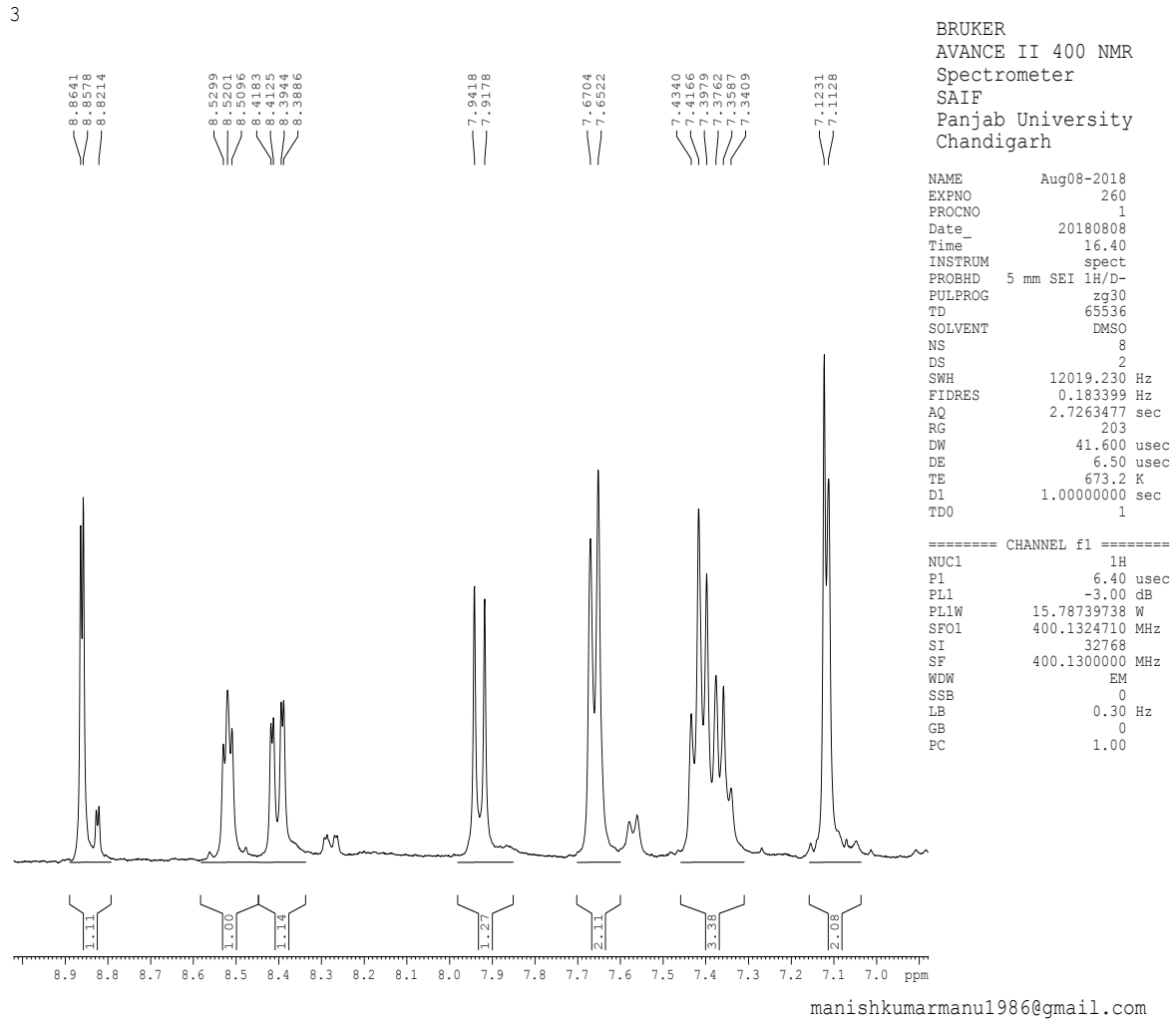


Figure 2: NMR spectra of [Cu $C_{70}H_{42}N_2O_{19})_2](SO_4)_2$] complex.

3.3. Electronic spectral studies

The UV-Visible spectra of Schiff bases and their copper complexes were recorded in MeCN solution at 300K. Ligands shows two peaks at 32054 and 24866 cm^{-1} due to intra ligand charge transfer (INCT) transition. Copper complexes shows a band at 19884 cm^{-1} and other strong broad band at 12568 cm^{-1}, which are assignable to $^2B_{2g}$- $^2B_{1g}$ and $^2B_{2g}$- $^2A_{1g}$ transitions which were appeared at 32847 and 24938 cm^{-1}region.In addition two INCT bands revealed that the copper complexes have distorted octahedral geometry.[32]

3.4. XRD studies

The X-Ray diffraction pattern of [Cu(L2)2].2H2O complex is given in fig 3 Single crystals of the complex could not be isolated from any solvents. The powder XRD pattern of Cu(II) complex show 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 [Cu(L2)]Cl2 complex is observed at 26.528°. All the peaks are fairly sharpened in the complex which indicate that the Quantum confinement of Schiff Base by the copper ion.[33] X- Ray diffraction pattern of copper chelate insist 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.[34]

$$D = 0.94\ \lambda/(\beta\ \cos\theta)$$

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.

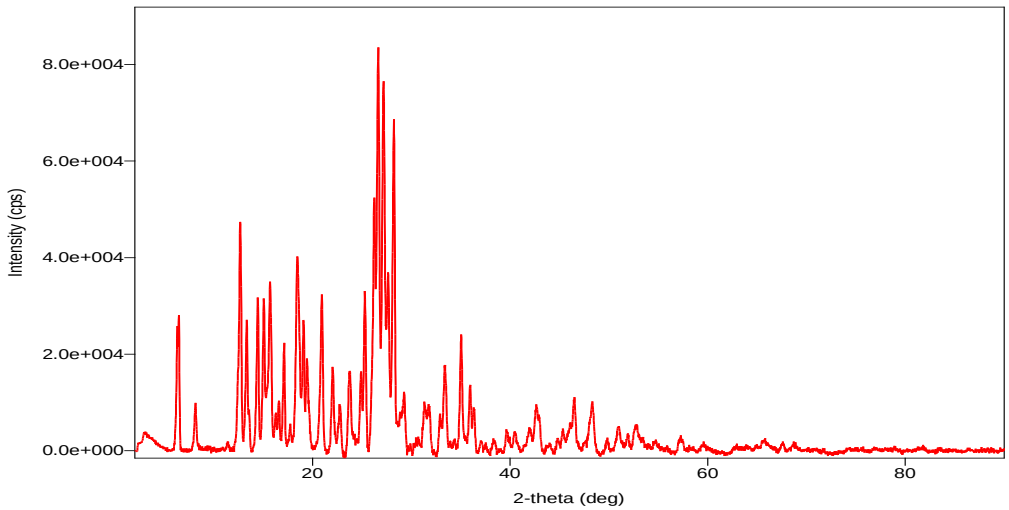


Figure 3: XRD spectra of [Cu $C_{70}H_{42}N_2O_{19})_2](SO_4)_2$] complex.

3.5 DPPH ACTIVITY

In DPPH free radical scavenging activity antioxidants are reacting with the stable free radical 1, 1 di phenyl picryl hydrazyl (DPPH) producing a colourless 1, 1 di phenyl-2-picryl-hydrazine. When DPPH receives an electron or hydrogen radical to become more stable, its absorbtion decreases.[35]

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 Cu(II) complexes are depicted in table--. Ascorbic acid was used as standard. The results(table--) illustrate the influence of Cu(II) complexes on the initiation DPPH antioxidant activity. These result clearly indicate that SL-1 and SL-4 copper complexes show higher antioxidant activities. The free radical scavenging activity of the compounds depends on the structural features.[36]

Table 3: IC_{50} values of DPPH radical scavenging activity of Cu(II) complexes Schiff base molecular adducts of doxorubicin.

S. NO.	COMPOUND	IC_{50} µG/ML
1	$[Cu\ C_{70}H_{42}N_2O_{19})_2](SO_4)_2]$	130.52 ± 3.76
2	$[Cu\ (C_{70}H_{42}N_2O_{19}Cl)_2](SO_4)_2]$	260.95 ± 0.66
3	$[Cu\ (C_{68}H_{40}N_2O_{21})_2](SO_4)_2]$	263.82 ± 2.38
4	$[Cu\ (C_{70}H_{44}N_2O_{19})_2](SO_4)_2]$	86.04 ± 1.57

Result showed that the scavenging activity were increased when increased the concentration of copper complexes.

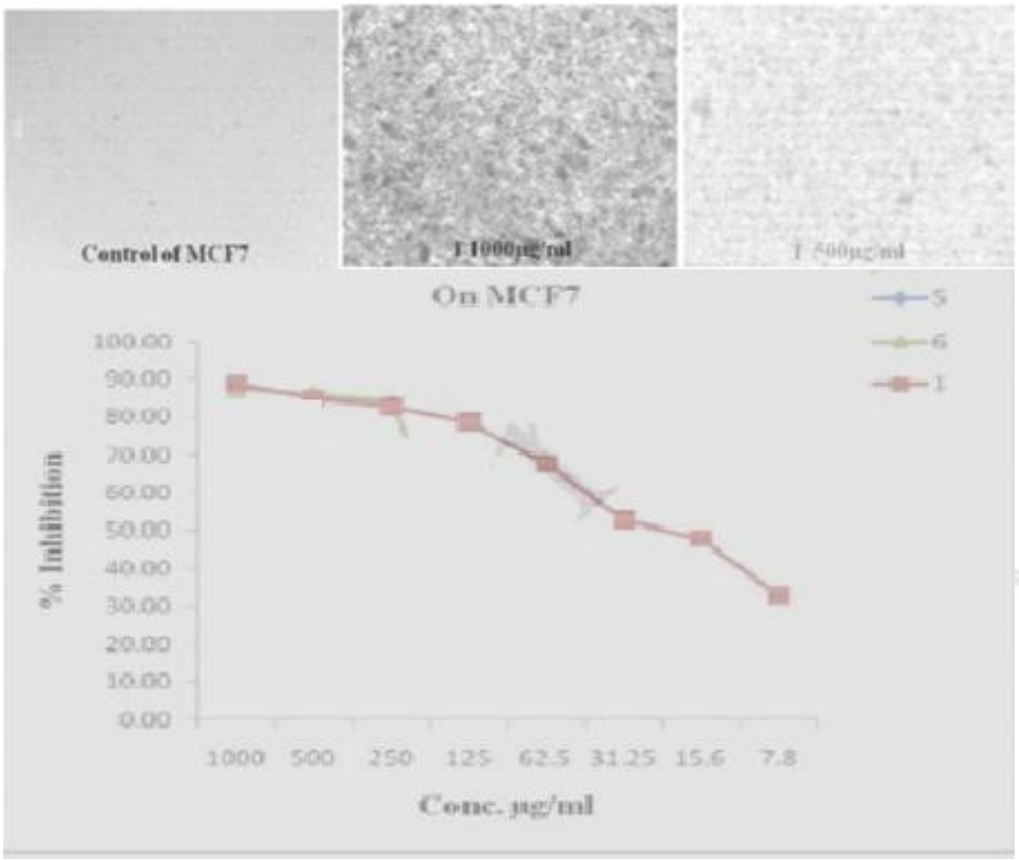
3.6 ANTICANCER ACTIVITY

The anticancer activity of the all Cu(II) complexes of Schiff base molecular adducts of doxorubicin were determined by MTT assay. The IC_{50} values of Cu(II) complexes are presented in table.4

Table 1: Invitro cytotoxic activity of copper complexes derived from Schiff base molecular adducts of doxorubicin drug.

S. No.	Name of Test Compound	Test Conc. (µg/ml)	% Cytotoxicity	CTC50 (µg/ml)
1.	$[Cu(DOX.L_{1)})_2](SO_4)_2$	1000 500 250 125 62.5 31.25 15.6 7.8	80.75±0.37 71.38±0.31 67.96±0.76 35.37±0.52 17.66±0.37 12.90±0.48 10.02±0.60 1.09±0.31	181.14±2.27
2.	$[Cu(DOX.\ L_2)_2](SO_4)_2$	1000 500 250 125 62.5 31.25 15.6	87.30±0.23 86.21±0.23 83.98±0.60 35.32±0.60 12.30±0.23 10.02±0.48 8.13±0.31	162.71±0.94

		7.8	5.26±0.87	
3.	$[Cu(DOX.\ L_3)_2](SO_4)_2$	1000 500 250 125 62.5 31.25 15.6 7.8	88.77±0.29 84.86±1.24 82.72±0.61 78.72±0.87 67.70±1.81 52.96±0.59 47.95±0.28 32.75±1.13	22.09±0.40
4.	$[Cu(DOX.\ L_4)_2](SO_4)_2$	1000 500 250 125 62.5 31.25 15.6 7.8	80.75±0.37 71.38±0.31 67.96±0.76 35.37±0.52 17.66±0.37 12.90±0.48 10.02±0.60 1.09±0.31	181.64±2.22



The pharmacological testing has proved that the cytotoxic effect of the Schiff base ligands and their Cu(II) Complexes was considerably moderate to less pronounced compared to the standard drug cisplatin, since calculated IC_{50} values were in the range of 22.09±0.40 to 181.64±2.22 µg/ml. among all ligands and their Cu(II) complexes evaluated, the Copper complex of HL3 showed the highest anticancer activity against MCF-7 (IC_{50}=22.09±0.40

µg/ml; IC_{50}= 1.9 µg/ml for cisplatin). Copper complex of HL1 showed the lowest IC_{50} value among all the ligands and their Cu(II) Complexes against MCF-7 Cell line. Furthermore copper complex of HL2 exhibited good cytotoxicity with concentration 162.71±0.94 µg/ml. Cu(II) complex of HL4 showed the moderate cytotoxicity with concentration 181.64±2.22 µg/ml against MCF-7 cell line. However the ligands have a higher inhibitory effect than their corresponding Cu(II) complexes. Several compounds in particular copper complexes of HL2 and HL3 were endowed with significant cytotoxic potency and can be considered as new lead compounds for further modification.

4. CONCLUSION

In summary, a series of Cu(II) complexes have been repared and characterized using various physical and spectroscopic techniques. All Cu (II) complexes showed considerable anticancer activity against MCF-7 cell lines and more pronounced antioxidant activity in the presence of DPPH. The cytotoxicity screening of the compounds revealed that, the selected compounds showed reasonable antitumor activity against MCF-7 cancer cell line in comparison to the traditional anticancer drug cisplatin. Among all tested compounds, 2 was found to have the highest inhibitory activity against MCF-7 cell lines with IC_{50} value of 22.09±0.40(µg/ml). have been synthesized and characterized by analytical, molar conductance, IR electronic magnetic susceptibility and powder XRD measurements and screened for various biological activities like antioxidants, and cytotoxicity. In Cu(II) complexes 1:2 copper to ligand molar ratio was obtained from analytical data. The molar conductance data confirm that complexes are non- electrolytic in nature. Based on the electronic and magnetic data, a distorted octahedral geometry is ascribed for the Cu(II) complexes. Synthesized Cu (II) complexes showed considerable invitro anticancer activity against MCF-7 cell lines and more pronounced antioxidant activity in the presence of DPPH. The copper complexes which act as superoxide radical (O_2^-) scavengers, is used in treating cancer by replacing the lost antioxidant activity which characterizes tumor systems. The low molecular weight and lipid solubility of the copper complexes facilitate penetration of cell membranes. Depending upon the specific type of copper complexes are used, treatment may result in decreased tumor growth, increased survival of the host organism, decreased tumor metastasis or induced morphological differentiation of cancerous cells. The complexes used according to the invention include copper complexes, their solvates as well as mixtures thereof. A method for the treatment of cancer using these copper complexes are also disclosed. The copper complexes, with the half-sandwich type structure, have demonstrated their potential

increasingly. Their coordination sites can be filled with various ligands, which offer numerous possibilities to modulate biological and pharmacological properties by proper ligand selection.

5. ACKNOWLEDGMENTS

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