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DESIGN, SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF COPPER MEDIATED MACROCYCLIC COMPLEXES

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ABSTRACT

A novel series of complexes of the type $[CuL_2]\ X_2$ where X = Cl^- , NO_3^-, SO_4^- were designed by molecular docking studies using PyRx 0.8 (Autodock vina based scoring function) and synthesized by template condensation of bis-hydrazone and thiosemicarbazide in methanolic medium. The complexes were characterized with the help of various physico-chemical techniques such as elemental analyses, molar conductance measurements, magnetic measurements and FT-IR, ^{1}H-NMR, LC-MS spectral studies. The low value of molar conductance indicates them to non-electrolyte in nature. Based on various studies, a distorted octahedral geometry may be proposed for all the complexes. All the synthesized macrocyclic complexes were also tested for their *in vitro* antibacterial activity against some pathogenic

bacterial strains. The MIC values shown by the complexes against these bacterial strains were compared with those of standard antibiotics streptomycin and tetracycline. Some of the complexes showed good antibacterial activities. The mono-anionic thiosemicarbazonate ligand act in a tridentate mode, binding through azomethine nitrogen and sulfur atoms. The cytotoxicity activity against breast cancer cell lines MCF-7 and antioxidant activities were evaluate for all the above compounds. All complexes were found to be highly active against the studied cell line; presenting the similar values of IC_{50} around 10 mmol/L.

Keywords: Estrogen Receptor α (ERα), Estrogen Receptor β (ERβ), Tamoxifen, Afimoxifene, Macrocyclic Copper (II) Complexes, Cytotoxicity.

INTRODUCTION

Design and chemistry of macrocyclic complexes has received much attention in recent years [1] due to their potential applications and importance in the area of medicinal chemistry [2-3]. Thus, the study of macrocyclic complexes is becoming a growing class of research[4]. Macrocycles are best prepared by the aid of metal ions as templates to direct the condensation reaction towards ring closure [5]. The field of macrocyclic chemistry of metals is developing very fast because of its variety of application[6] and importance in the area of inorganic medicinal chemistry [7]. The rational design and construction of inorganic and organometallic metallomacrocycles by transition metal-directed multi-component self assembly has a major impact on pharmacological activity.[8-9]

The incorporation of metal centers into supramolecular system gives rise to novel electronic and/or magnetic properties as well as fascinating structural features. Copper (II) is the most studied metal ion among all the transition metal ions [10-11] Cu(II) complexes are known to play a significant role either in naturally occurring biological systems or as pharmacological agents [12-13]. In recent years, several families of copper complexes have been studied as potential antitumor agents. Therefore, molecular docking studies of this copper complex was done on Estrogen Receptor α, Estrogen Receptor β and compared with Tamoxifen and Afimoxifene. Results of copper complex was highly significantly, thus led to synthesis, characterization and pharmacological evaluation of compounds.

MATERIALS AND METHODS

Accession of Target Protein: The three-dimensional structures of Estrogen Receptor α (ERα, PDB ID: 3DT3) and Estrogen Receptor β (ERβ, PDB ID: 2QTU) were downloaded from the RCSB protein Data Bank in pdb format. [14,15]

Selection of Ligands: Chemical structure of Copper complex was prepared by Marvin sketch. Chemical structures of Tamoxifen and Afimoxifene were prepared by using Chem BioDraw Ultra 12.0 and Chem 3D Ultra 8.0 and saved in pdb format.

Optimization of Target Protein and ligands: Two proteins (ER α and ER β) have bound ligands attached, which were deleted by using Discovery Studio 4.5. PDB PDB Coordinates of target protein were optimized by using Discovery Studio 4.5 ad UCSF Chimera 1.10.2. and saved as a pdb file. Copper complex was optimized by Marvin Sketch. Tamoxifen and Afimoxine were optimized by Chem 3D Ultra 8.0 using energy minimization process.

Docking Analysis

A computational approach of ligand-protein docking was done to analyze the binding scores and interactions. Docking was done by PyRx 0.8 which uses autodock vina based scoring function.[14] "Grid point" has been assigned for each with respect to ERα and ERβ each. After getting binding scores, respective files were uploaded to Pymol 1.1 and Discovery Studio 4.5 for knowing the different protein-ligand interactions and amino acids involved.

RESULTS AND DISCUSSION

Docking results of copper complex, tamoxifen and afimoxifene on both Estrogen Receptor α (ERα) and Estrogen Receptor β (ERβ) are given in Table 1 and Table 2. The respective docking images are given in Figure 1-17[16]

Table 1: Docking results of copper complex, tamoxifen and afimoxifene on ERα

Sl. No.	Compound	BindingAffinity (kcal/mol)	RMSD Upper bound	RMSD Lower bound	Amino acids involved in H-bonding
1	Copper complex	-9.9	0.0	0.0	Thr 278 Asp 282
4.	Tamoxifen	-9.4	0.0	0.0	-
5.	Afimoxifene	-9.6	0.0	0.0	Arg 82

Table 2: Docking results of copper complex, tamoxifen and afimoxifene on ERβ

Sl. No.	Compound	BindingAffinity (kcal/mol)	RMSD Upper bound	RMSD Lower bound	Amino acids involved in H-bonding
1.	Copper complex	-9.4	0.0	0.0	Glu 109 Glu 286 Ser 287 Ser 363
2.	Tamoxifen	-6.4	0.0	0.0	Ser 287
3.	Afimoxifene	-6.5	0.0	0.0	Ser 287

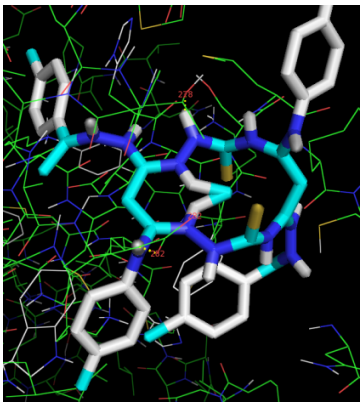
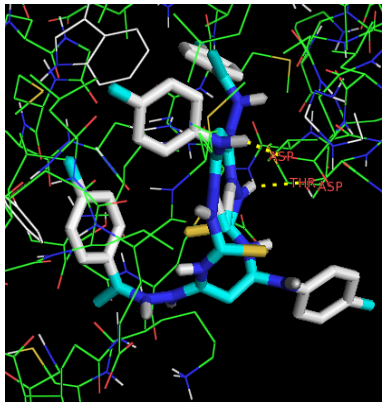
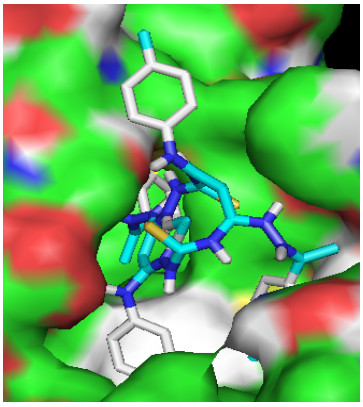




Figure 1 **Figure 2** **Figure 3**

Figure 1-3: Copper complex within Estrogen Receptor α

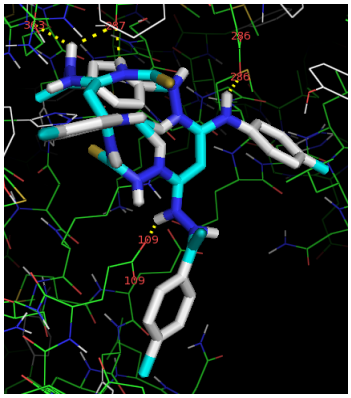
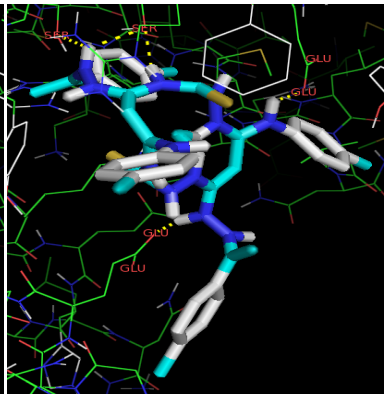
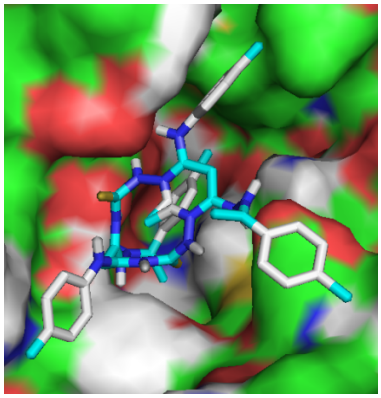




Figure 4 **Figure 5** **Figure 6**

Figure 4-6: Copper complex within Estrogen Receptor β

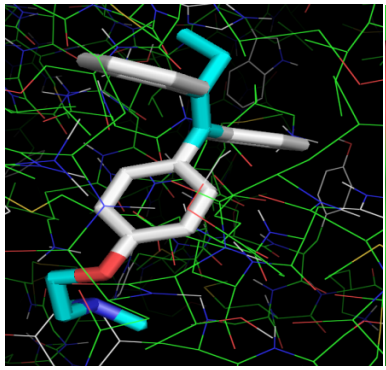
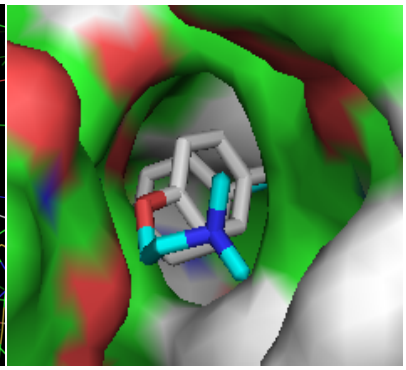



Figure 7 **Figure 8**

Figure 7-8: Tamoxifen in Estrogen Receptor α

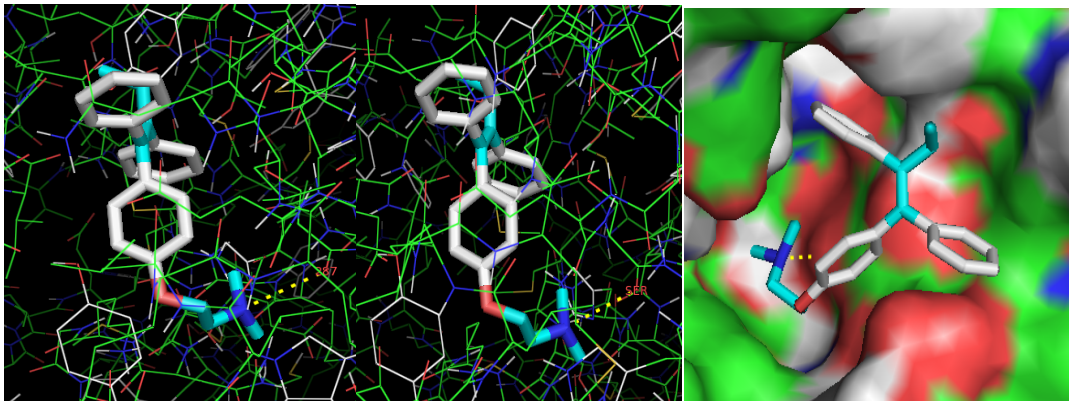


Figure 9 **Figure 10** **Figure 11**

Figure 9-11: Tamoxifen within Estrogen Receptor β

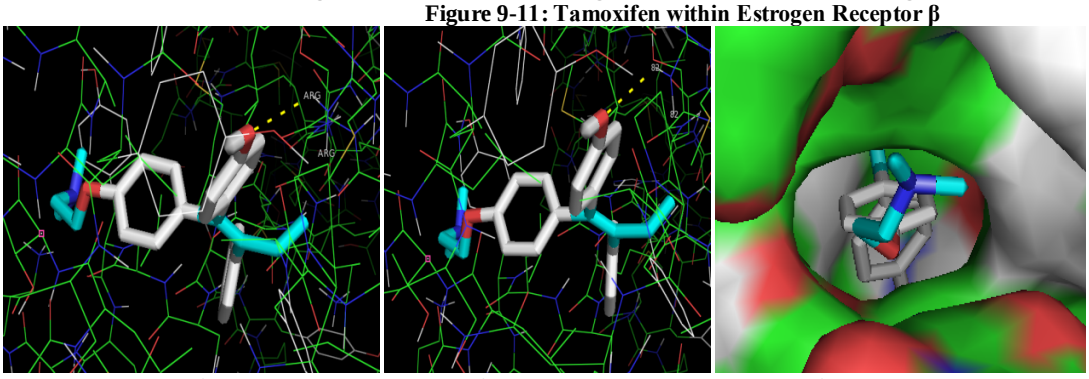


Figure 12 **Figure 13** **Figure 14**

Figure 12-14: Afimoxifene within Estrogen Receptor α

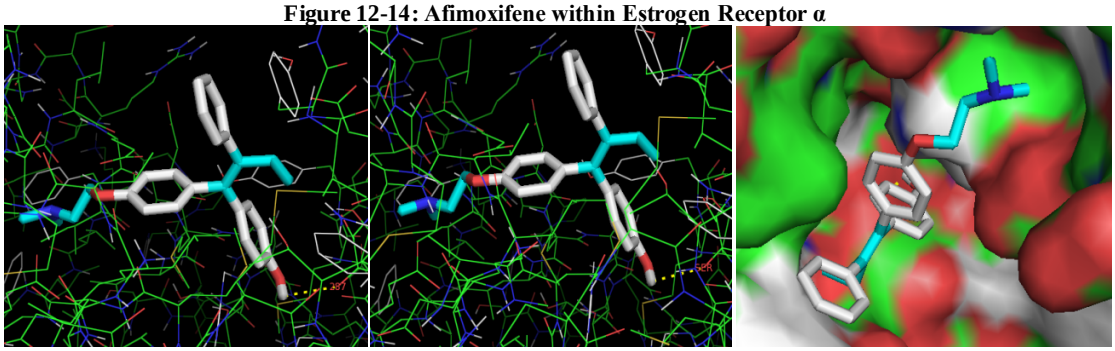


Figure 15 **Figure 16** **Figure 17**

Figure 15-17: Afimoxifene with Estrogen Receptor β

Synthesis of Complexes: All the materials, chemicals and solvent used in this study were of analytical grade. Thiosemicarbazide, copper salts were purchased from S.D. fine, Merck, and Ranbaxy and were used as received.

All the complexes were synthesized by the template method i.e. by condensation of bis-hydrazone and thiosemicarbazide in the presence of the divalent copper salts. To a stirred hot methanolic solution (≈ 50 cm^3) of thiosemicarbazide (2 mmol) and bis-hydrazone were added to a divalent copper salts (1 mmol) dissolved in the minimum quantity of methanol. The resulting solution was refluxed for 8 – 10 hrs. The mixture was

concentrated to half its volume and kept in desiccators overnight. On overnight cooling, a dark colored precipitate formed which was filtered, washed with methanol and dried in vaccuo. The obtained yield was 40-60%. The complexes were soluble in DMF and DMSO. They were found to be thermally stable upto 260-280° C, after which decomposition occurred.

Analytical and Physical measurements: Microanalysis for (C, H and N) were performed using an elemental analyser for copper (II) complexes. The metal content in the copper contents in the complexes were determined by a literature method [17]. The IR spectra were recorded on a FT-IR spectrophotometer (Perkin Elmer) in the range 4000-200 cm^{-1} using nujol mull. The H^1NMR spectra (at room temperature, DMSO – d6) were recorded on a Bruker AVANCE II 400 NMR spectrometer (400 MHz) at the SAIF, Punjab University, Chandigarh. The electronic spectra (in DMSO) were recorded on a cary 14 spectrophotometer at room temperature. The FAB (Fast atom bombardment) mass spectra (at room temperature) were recorded on a TOF MS ES^+ mass spectrometer. The conductivity was measured using a digital conductivity meter (HPG system G- 3001). The melting points were determined in capillaries using an electrical melting point apparatus.

In-vitro **antibacterial activity:** All synthesized macrocyclic complexes were tested for invitro antibacterial activity against some bacterial strains using Muller – Hinton agar [18]. Four test pathogenic bacterial strains, viz. E. Coli, Bacillus subtilis, S. Aureus and P. aeruginosa were considered for the determination of the MICs (minimum inhibitory concentration) of macrocyclic dioxime for (I, II and III) complexes. The sterilized (autoclaved at 121 ° C for 15 min) medium (40-50 °C) was poured into the petri dishes to give a depth of 3-4 mm and allowed to solidify. The suspension of the microorganism streaked on plates. The paper discs were placed on the solidified medium. The plates were incubated for 1 hrs at room temperature and incubated at 37°C for 24 hrs [19].

In-vitro **anti tumor activity:** For Cytotoxicity assays, the cells were plated at $1x10^4$ cells/well in 96-well plate, and grown in DMEM supplemented with 10% FBS for 48 hrs before treatment. The cells were treated with various doses of compounds (1-25μM) for 48 hrs followed by Sulforhodamine–B (SRB) performed as reported earlier [20-21]. Briefly, at the end of the experiment, the cells were fixed with 10% TCA for 1 h at 4^oC. The supernatant was aspirated; plates were washed with

deionized water 3 times and air-dried. 50µl of 0.4% (w/v) SRB in 1% acetic acid was added to each well and incubated for 30 min at room temperature. Unbound SRB was removed by 3 washes with 1% acetic acid and the plates air-dried. 200 µl of unbuffered 10mM Tris base, pH 10.5 was added for extracting the bound stain. The absorbance was read at 560nm in a SpectraMax Me2 Elisa Microplate Reader (Molecular Devices Inc.). Suitable untreated controls were also concomitantly employed. For the morphological analysis, $0.2x10^6$ cells/well were plated into 6-well plate in DMEM medium supplemented with 10% FBS for 48 hrs before treatment. The cells were treated with various doses of compounds (0.1-20 µM) for the next 48 hrs and at the end of experiment cells were observed under phase contrast microscope & photographed (Nikon Eclipse Ti, Japan).

Antioxidant activity: The ability of synthetic compounds to scavenge hydrogen peroxide was determined as reported previously. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentages of hydrogen peroxide scavenging of H_2O_2 by compounds were calculated as follows:

% Scavenged [H_2O_2] = [(AC – AS)/AC] x 100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the compounds [22-23].

All the complexes are stable to the atmosphere. The complexes are soluble in DMF and DMSO and partial soluble in methanol. The elemental analyses are consistent with the proposed structure of the complexes. Conductivity measurements in DMSO indicated them to be non-electrolyte. The tests of the anions were negative only after decomposition of the complexes, indicating their presence outside the coordination sphere. The analytical data of the reported complexes are given in table 3.

IR Spectra: The presence of a single medium band in the region 400 - 4000 cm^{-1} in all the complexes may be assigned to N-H stretching vibrations and relevant IR spectra of complexes are given in table 4.

Table 3: Analytical data of the copper complexes

S N	Molecular formula & molecular weight	Color	Yield	M.P. (°C)	Molar Conduc-tance ($\Omega^{-1}cm^2$ mol^{-1})	% M	% C	%H	% N

1	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]Cl_2$	Greenish Yellow	52 %	230	57.5	7.26/ (7.32)	49.56/ (49.80)	3.98/ (4.15)	14.1/19.36
2	$[Cu(C_{36}H_{36}N_{12}O_2S_2)](NO_3)_2$	Greenish	39 %	155-160	127.2	6.80/ (6.90)	46.86/ (46.93)	3.81/ (3.91)	20.9/21.29
3	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]SO_4$	Light Green	59 %	220	10.7	7.25/ (7.28)	48.75/ (48.90)	4.10/ (4.13)	21.5/(21.8)

Table 4: Relevant IR spectra of the copper complexes (cm^{-1})

S.No.	Copper Complex	ν (N-H)	ν (Ar-H)	ν(C=N)	ν(C=S)	ν (M-N)
1	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]Cl_2$	3310	3061	1620	1170	435
2	$[Cu(C_{36}H_{36}N_{12}O_2S_2)](NO_3)_2$	3305	3065	1625	1176	445
3	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]SO_4$	3300	3071	1628	1189	455

It was noted that a pairs of bands corresponding to ν (NH_2) stretching vibrations appeared at 3200-3210 cm^{-1} in the IR spectrum of thiosemicarbazide but was absent in the IR spectra of all the copper complexes. Furthermore, no strong absorption band was observed in the spectra of the complexes near 1700 cm^{-1} indicating, the absence of the (C=O) group of carbohydrazone and thus combining the condensation of the carbonyl group of carbohydrazone and the amino group of thiosemicarbazide. A strong absorption band in the region 1595-1625 cm^{-1} may be assigned to C=N stretching vibrations [24-25]. These results provide strong evidence for the formation of the macrocyclic frame[23]. The lower values of (C = N) may be explained based on a drift of the lone pair electron density of the azomethine nitrogen towards the central metal atom[26-27]. Another set of medium intensity bands in the region 1500-1585 cm^{-1} were attributed to ν (c=c) aromatic stretching vibrations of the phenyl groups and the bands around 845-875 cm^{-1} may be assigned C-H out of plane bending vibrations of the phenyl groups. The C-N stretching vibration may occur in the range 1015-1355 cm^{-1}.The far IR spectra of the complexes showed bands in the region 422-435 cm^{-1} corresponding to ν(M-N) stretching vibrations[28-29] which give insight into the coordination of the azomethine nitrogen to the central copper atom [30].

^{1}H-NMR Spectra: The ^{1}HNMR spectra of all complexes were obtained in the $CDCl_3$ at room temperature using TMS as an internal standard. The aromatic region shows a sharp singlet at δ 7.40 ppm assigned to the phenyl protons and a singlet at δ2.55 ppm due to methyl protons. The O-H proton of a phenolic group shows a sharp singlet at δ11.47 ppm. The multiplets observed in the region 6.81-7.93 ppm may be assigned to the aromatic ring protons of carbohydrazone and the thiosemicarbazide moiety. The H^1NMR spectra of copper complexes shows signals corresponding to $–CH_3$, $-NH_2$, NH (hydrazone) and -OH protons at 2.28 (5, 3H), 7.40-7.48 (M, 3H), 8.059-8.38 (2H), 10.09 (s,

1H) and 11.83 (s, 1H) respectively. The NMR spectrum of copper chelates confirms the participation of $–NH_2$ group and imino – NH group in the coordination with metal ions. (Figure 5)

Table 5: Relevant H^1NMR peaks of copper complexes (ppm)

S.No.	Complex	δ (CH_2)	δ (NH)	δ(HC=N)	δ(CH_3)	δ(ArCH)
1	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]Cl_2$	2.45	9.89	8.35	2.2	6.7
2	$[Cu(C_{36}H_{36}N_{12}O_2S_2)](NO_3)_2$	2.4	9.99	8.2	2.1	6.8
3	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]SO_4$	2.5	9.96	8.06	2.1	6.5

Some hydrogen atom values of δ were not observed precisely due to overlapping with the signals of the aromatic hydrogen atoms of carbohydrazone ligand. ^{1}HNMR integrations and signal multiplicity are in agreement with the proposed structures. In the ^{1}HNMR spectra of the complexes a high frequency shift of Ca 0.13 ppm, for the methyl hydrogen atoms (C-CH_3), compared to the spectra of the thiosemicarbazones, and evidences the coordinator through the azomethine nitrogen atom. Electronic spectra of Cu (II) complexes exhibit bands in the range 15270 – 16680 cm^{-1} and 18,200 – 19200 cm^{-1} respectively.

Magnetic measurements and electronic spectra: The magnetic moments of copper complexes were found in the range 1.75-1.83 μB corresponding to one unpaired e^- in the copper (II) ion. The absorption spectra of the copper complexes exhibited bands in the region 17700-19680 cm^{-1} which showed that these complexes were distorted octahedral in nature [31-32]. Assuming tetragonal distortion in the molecule, the d – orbital energy level sequence for these complexes may be represented as $x^2 – Y^2 > Z^2 > XY > XZ > YZ$ and the shoulder may be assigned to $Z^2 \rightarrow x^2 – Y^2$ ($^2B_{1g} \rightarrow {}^2B_{2g}$) and the broad band contains both the $XY \rightarrow x^2 – Y^2$ ($^2B_{1g} \rightarrow {}^2Eg$) and XZ, $YZ \rightarrow x^2 – Y^2$ ($^2B_{1g} \rightarrow {}^2A_{2g}$) transitions [33]. The band separation of the spectra of the complexes was of the order of 2500 cm^{-1}, which is consistent with the proposed geometry of these complexes. Therefore it may be concluded that all the complexes of copper (II) have a distorted octahedral geometry.

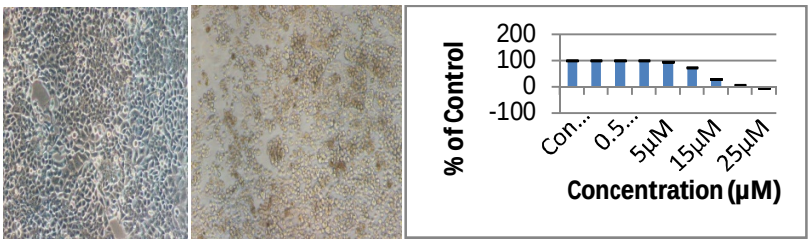
Cytotoxic Activity: All the synthesized macrocyclic Cu(II) complexes were evaluated for their effectiveness against the breast cell line MCF – 7. For comparison purpose, the cytotoxicity of cis platin was evaluated under the same experimental condition. The values of cell viability were calculated after the tested compounds were incubated for 48 hrs. (The LD50 values, calculated from the close survival groups from MTT assay, are shown in table 6) Comparing only the values of LD_{50} of all complexes, the order of cytotoxic activity [Cu(C36H36N12O2S2)](NO3)2 >

[Cu(C36H36N12O2S2)]SO4 > [Cu(C36H36N12O2S2)]Cl2 complexes. The good values of activity found for these complexes, around 10 µ mol/L, show that the complexation of thiosemicarbazone to Cu (II) may be a good strategy to obtain antitumor agents. (Figure 18-20). The similarity of the values of LD_{50} found for the Cu(II) complexes is an evidence in favour of the same biochemical action mechanism, but different from those of the cisplatin, inactive in this case. Three macrocyclic copper (II) complexes were screened for their potential anticancer/cytotoxic activity in table no. 6.

***In-vitro* antibacterial activity:** The three macrocyclic Cu(II) complexes were also evaluated for their potential antibacterial activity against B. Subtilis, S. aureus , E. Coli and P. aereoginasa. Table 5-7 highlight the antibacterial activity against B. Subtilis, S. aureus , E. Coli as observed by dise diffusion method. None of the compounds were found to be active against P. aereoginasa at any concentration.

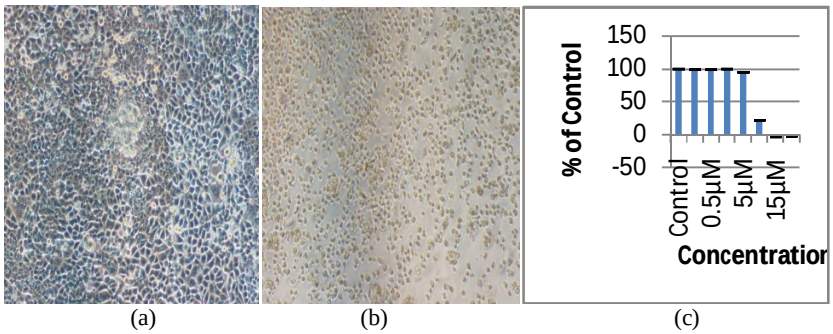
Table 6: *In-vitro* cytotoxicity activity of copper complexes

S. No.	Complex	R	LD_{50} value
1	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]Cl_2$	$R_1 = CH_3$, $R_2 = Cl$, and $R_3 = OH$	15 µM
2	$[Cu(C_{36}H_{36}N_{12}O_2S_2)](NO_3)_2$	$R_1 = CH_3$, $R_2 = NO_3$, and $R_3 = OH$	10 µM
3	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]SO_4$	$R_1 = CH_3$, $R_2 = SO_4$ and $R_3 = OH$	10 µM



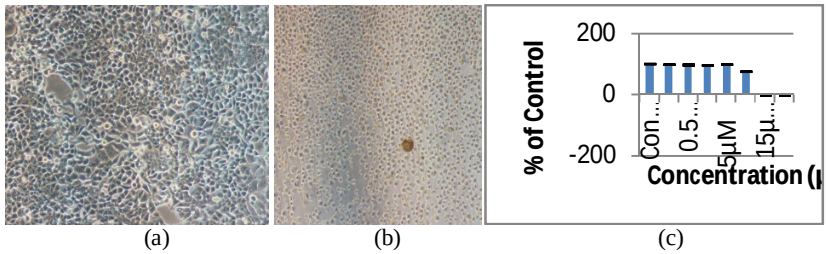
(a) (b) (c)

Figure 18: (a) Control showing cancer cell line MCF-7 (b) Cytotoxic activity of compound 1 at 15µM (c) Graph showing the Dose dependent effect of compound 1 on MCF-7



(a) (b) (c)

Figure 19: (a) Control showing cancer cell line MCF-7 (b) Cytotoxic activity of compound 2 at 15µM (c) Graph showing the Dose dependent effect of compound 2 on MCF-7



(a) (b) (c)

Figure 20: (a) Control showing cancer cell line MCF-7 (b) Cytotoxic activity of compound 3 at 15µM (c) Graph showing the Dose dependent effect of compound 3 on MCF-7

Table 7: *In-vitro* antibacterial activity of compounds 1-3 against *B. subtilis*

Compound	Ring Diameter (mm)						
	Tetracyclin (1mg/ml)	DMSO (1mg/ml)	Compound Dose (mg)				
			2	4	6	8	10
1.	20	ND	ND	ND	ND	6	ND
2.	20	ND	ND	ND	ND	ND	ND
3.	15	ND	ND	ND	ND	ND	ND

Table 8: *In-vitro* antibacterial activity of compounds 1-3 against *S. aureus*

Compound	Ring Diameter (mm)						
	Tetracyclin (1mg/ml)	DMSO (1mg/ml)	Compound Dose (mg)				
			2	4	6	8	10
1.	15	ND	17	14	ND	ND	ND
2.	18	ND	10	11	8	7	16
3.	15	ND	12	6	6	12	16

Table 9: *In-vitro* antibacterial activity of compounds 1-3 against *E. coli*

Compound	Ring Diameter (mm)						
	Tetracyclin (1mg/ml)	DMSO (1mg/ml)	Compound Dose (mg)				
			2	4	6	8	10
1.	ND	ND	6	6	6	8	ND
2.	15	ND	6	6	6	16	12
3.	ND	ND	8	8	8	10	11

The minimum inhibitory concentrations of complexes were determined by disc diffusion method. The minimum inhibitory concentration at which no growth observed was taken as MIC values. None of the compounds active against P. aerogenosa at any concentration. The higher antibacterial activity of the copper (II) complexes may be due to coordination and chelation tends to make copper complexes act as more powerful and potent bacteriostatic agents thus inhibiting the growth of the bacteria. In a complex, the positive charge of the copper is partially shared with the donor atoms present in the complexes and these may be Π electrons delocalization over the whole. The increase activity of the copper chelate can be explained on the basis of chelation theory. On chelation, the polarity of the copper ion if reduced largely due to the overlap of the ligand orbital and the partial sharing of the positive charge of the metal ion with the donor groups. This increases the lipophilic character of these complexes seems to be the reason of their enhanced potent antibacterial activity. There are some other factors which

also increase the activity such as solubility, conductivity and bond length between the metal and the ligand. All three copper complexes were active due to the presence of thio group in the coordinating ligand.

***In-vitro* antioxidant activity:** Macrocyclic and their metal complexes have been suggested as promising agents for the diagnosis and treatment of different disease [34-36]. All three compounds showed significant free radical scavenging action against hydrogen peroxide (H_2O_2) induced release of free radicals at different concentration 200, 400, 800 and 1000 µg/ml. Ascorbic acid used as reference standard.

All compounds were found to possess potent antioxidant activity in the range of 80-90 %. When screened for their radical scavenging activity against H_2O_2.

Table 10: *In-vitro* antioxidant activity of copper complexes

S.No.	% Scavenging (Mean ± SEM) of triplication				
	Compound	200 µg/ml	400 µg/ml	800 µg/ml	1000 µg/ml
1	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]Cl_2$	26.22 ± 0.082	31.26 ± 0.176	33.62 ± 0.210	40.64 ± 0.094
2	$[Cu(C_{36}H_{36}N_{12}O_2S_2)](NO_3)_2$	33.18 ± 0.034	36.94 ± 0.022	37.60 ± 0.091	42.62 ± 0.090
3	$[Cu(C_{36}H_{36}N_{12}O_2S_2)]SO_4$	36.16 ± 0.042	35.94 ± 0.026	36.17 ± 0.042	35.92 ± 0.021
4	Ascorbic acid (Standard)	40.16 ± 0.022	39.69 ± 0.024	42.26 ± 0.014	40.19 ± 0.012

CONCLUSION

Based on molecular docking studies and other various studies like elemental analysis, conductance measurements and magnetic susceptibilities, as well as IR, H^1NMR, electronic and mass spectral studies, a distorted octahedral geometry may be proposed for all these complexes. The proposed structures are shown in figure 21-23.

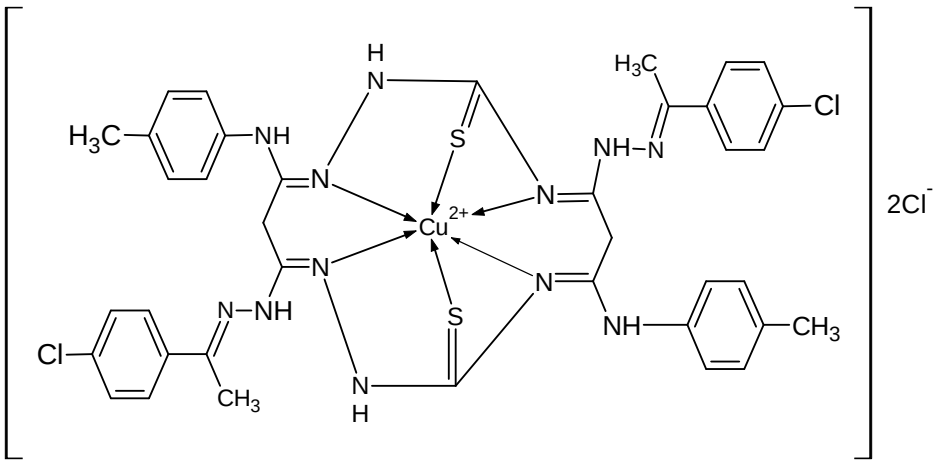


Figure 21: Structure of copper complex 1

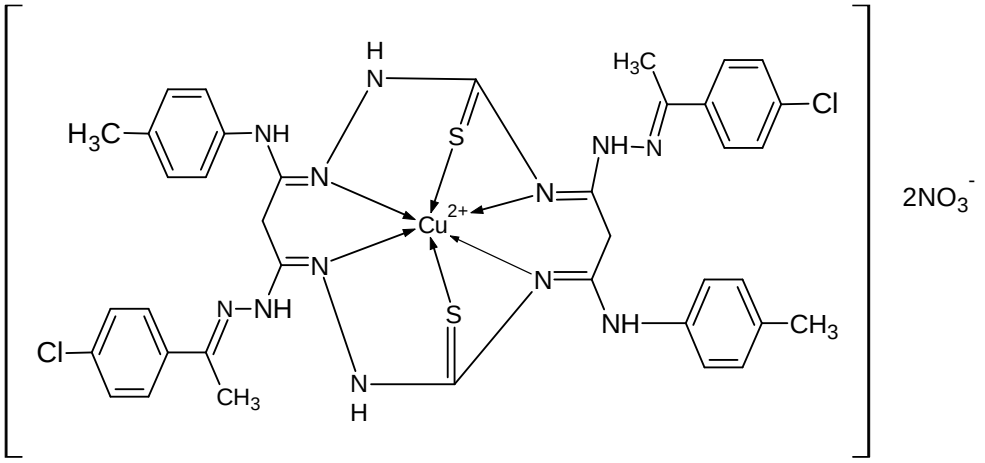


Figure 22: Structure of copper complex 2

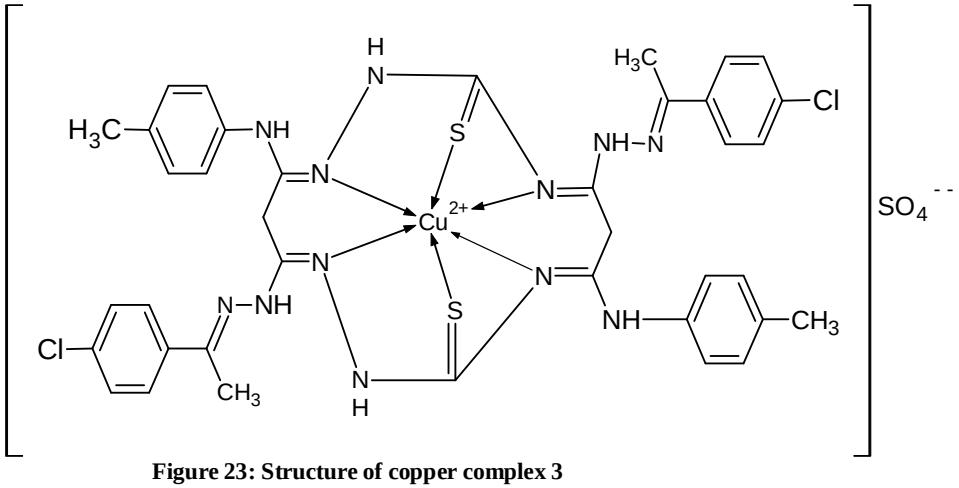


Figure 23: Structure of copper complex 3

These copper complexes have shown excellent molecular docking studies by giving significant binding energies i.e. -9.9 kcal/mol and -9.4 kcal/mol on Estrogen Receptor α and Estrogen Receptor β respectively and compared with two anticancer drugs used in breast cancer i.e. Tamoxifen and Afimoxifene.

These three Cu(II) complexes show modest in-vitro cytotoxic properties against breast cancer cell line MCF- 7. LD 50 values are compared with cisplatin and the results revealed that complex possess better activity. More detailed studies are needed to understand the mechanism of action at the cellular level and the role of the metal.

Investigations of antibacterial screening data revealed that the compounds 1, 2 and 3 exhibited maximum zone of inhibition against the bacterial strains *E. coli*, *S. aurous, B. Subtilis.* It has been suggested that chelation reduces the polarity of the metal ion, mainly because of the partial sharing of its positive charge with a donor group within the whole chelate ring system. This process of chelation increases the lipophilic nature of

the central metal atom, which in turn, favour its permeation through the lipid layer of the membrane, thus causing the metal complexes to cross the bacterial membrane more effectively thereby increasing the activity of the complexes. In addition to this many other factors, such as solubility, dipole moment and conductivity as well as the influence of the metal ion, may be possible reasons for the remarkable antibacterial activities of these complexes. Analysis of result revealed that the all three macrocyclic Cu(II) complexes exhibited good radical scavenging activity as compared to the standard ascorbic acids. Apparently, potency of all three complexes were found to be relatively low to cis-platin compounds which are capable of inducing cell death via apoptosis are regarded as potent anticancer drugs. Cell shrinkage and rounding, membrane bubbling, chromatin condensation and nuclear fragmentation are important characteristics of apoptosis. In our study, prominent morphological changes, which are associated with apoptosis, live cell rounding and shrinkage and nuclear fragmentation were observed when MCF – 7 breast cancer cell line were treated with the macrocyclic Cu(II) complexes(10 hrs) for more potent 24 hrs. The data reported in this paper may be helpful guide for the medicinal chemist who is working in this area.

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REFERENCE

[1] Chaudhary A, Bansal N, Fahmi N, Singh RV, Tetraazamacrocycles of manganese and iron metals: Synthesis, spectroscopic characterization and biological evaluation, Indian J. Chem., 2004, 43, 320-322.

[2] Chandra S, Sharma SD, Chromium(III), manganese(II), cobalt(II), nickel(II), copper(II) and palladium(II) complexes of a 12-membered tetraaza [N_4] macrocyclic ligand, Transition Met. Chem., 2002, 27, 732-735.

[3] Chandra S, Kumar R. Synthesis and spectral studies on mononuclear complexes of chromium(III) and manganese(II) with 12-membered tetradentate N_2O_2, $N_2S_2$and N_4 donor macrocyclic ligands. Transition Met. Chem., 2004, 29, 269-275.

[4] Chandra S, Gupta R, Gupta N, Bawa SS, Biologically Relevant Macrocyclic Complexes of Copper Spectral, Magnetic, Thermal and Antibacterial Approach. Transition Met. Chem., 2006, 31, 147-151.

[5] Singh AK, Singh R, Saxena P., Trasition Met. Chem., 2004, 29, 864.

[6] Hosseini MW, Lehn JM, Duff SR, Gu K, Mertes MP, Synthesis of mono- and difunctionalized ditopic [24]N_6O_2 macrocyclic receptor molecules, J. Org. Chem., 1987, 52(9), 1662-1666.

[7] Izan RM, Brandshaw JS, Nielsen SA, Lamb JD, Christensen JJ, Sen D, Chem. Rev., 1985, 85, 271.

[8] Jones CJ. Chem. Soc. Rev., 1998, 27, 289.

[9] Philp D, Stoddart JF, Anrew. Chem., 1996, 108, 1242

[10] Ajibade PA, Zulu NH, Metal complexes of diisopropylthiourea: Synthesis, Characterization and antibacterial studies. Int. J. Mol. Sci., 2011, 12, 7186-7198.

[11] Reddy PM, Prasad AVSS, Ravinder V, Synthesis, Spectral Characterization, Catalytic and antibacterial activity of macrocyclic Cu (II) compounds, Transition. Met. Chem., 2007, 32, 507-513.

[12] Budige G, Puchakayala MR, Kongara SR, Hu A, Vadde R, Synthesis, Characterization and Biological evaluation of mononuclear Co(II), Ni(II), Cu(II) and Pd(II) complexes with new N_2O_2 Schiff base ligands. Chem., Pharm. Bull., 2011, 59, 166-171.

[13] Chandra S, Jain D, Sharma AK, Sharma P, Coordination modes of a Schiff base pentadentate derivative of 4-aminoantipyrine with Cobalt(II), Nockel(II) and Copper(II) metal ions: Synthesis, Spectroscopic and antimicrobial studies, Molecules 2009, 14, 174-190.

[14] Fang J, Akwabi-Ameyaw A, Britton JE, Katamereddy SR, Navas F, Miller AB. et al., Synthesis of 3-alkyl naphthalenes as novel estrogen receptor ligands. Bioorg. Med. Chem. Lett., 2008, 18, 5075-5077.

[15] Richardson TI, Dodge JA, Wang Y, Durbin JD, Krishna V, Norman BH, Benzopyrans as selective estrogen receptor beta agonists (SERBAs). Part 5: Combined A- and C-ring structure-activity relationship studies, Bioorg. Med. Chem. Lett., 2007, 17, 5563-5566.

[16] Trott O, Olson AJ, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, J. Comput. Chem. 2010, 31, 455-461.

[17] A.I. vogel's A text book of quantitative chemical analysis, 5th ed., Longmau, London, 1989.

[18] Singh DP, Kumar R, Singh J, Eur. J. Med. Chem., 2009, 44, 1731.

[19] Selvam PP, Priya MG, Kumar NR, Saravanau GI, Indian J. Pharm. Sci., 2009, 71, 428.

[20] Nigam M, Ranjan V, Srivastava S, Sharma R, Balapure AK, Centchroman induces G0/G1 arrest and caspase-dependent apoptosis involving mitochondrial membrane depolarization in MCF-7 and MDA MB-231 human breast cancer cells, Life Sci. 2008, 82, 577-590.

[21] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D. et al., New colorimetric cytotoxicity assay for anticancer-drug screening., J. Natl. Cancer Inst., 1990, 82, 1107-1112.

[22] Ruch RJ, Cheng SJ, Klaunig JE, Prevention of cytotoxicity and inhibition of intracellular communication by antioxidants catechins isolated from Chinese green tea., Carcinogenesis 1989, 10, 1003-1008.

[23] Tiwari AK, Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidants therapy, Curr. Sci., 2001, 81, 1179-1187.

[24] Singh AK, Singh R, Saxena P. Transition Met. Chem., 2004, 29, 867.

[25] Gupta LK, Chandra S. Transition Met. Chem. 2006, 31, 368.

[26] Zeng Q, Sun J, Gou S, Zhou K, Fang J. Transition met. chem., 1998, 23, 371.

[27] Lodeiro C, Bastida R, Bertolo E, Macias A, Rodriguez R. Transition met. chem., 2003, 28, 388.

[28] Shakir M, Islam KS, Mohamed AK, Transition met. chem., 1999, 24, 577.

[29] Aqra FMAM., transition met. chem., 1999, 24, 337.

[30] Rana VB, Singh DP, Singh P, Teolia MP, Transition met. chem., 1982, 7, 174.

[31] (a) Rana VB, Singh DP, Singh, P, Teotia MP, Transition met. Chem., 1981, 6, 36.

(b) Rana VB, Singh DP, Singh, P, Teotia MP, Polyhedron 1982, 1, 77.

[32] Lever ABP, Inorganic electronic spectroscopy, Amsterdam, Elsevier, 1968.

[33] Lever ABP, Mantovani E. Inorg. Chem., 1971, 10, 817.

[34] Liang X, Sadler PJ. Chem. Soc. Rev., 2004, 33, 246-266.

[35] Marques F, Gano L, Campello MP, Laceida S, Santos I, Lima LM. et al., J. Inorg. Biochem., 2000, 100, 270-280.

[36] Marques F, Guerra KP, Gano L, Campello MP, I Santos, Lima LM. et al., J. Biol. Inorg. Chem., 2004, 9, 859-872.