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SYNTHESIS, CHARACTERIZATION, ANTICANCER, ANTIBACTERIAL AND ANTIOXIDANT EVALUATION OF MACROCYCLIC COPPER (II) COMPLEXES DERIVED FROM THIOSEMICARBAZIDE.

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ABSTRACT

Three Cu (II) complexes were prepared from N_4 substituted thiosemicarbazones [Cu (p-clbhtsc)$_2$]Cl_2.2H_2O [1], [Cu (p-mbhtsc)$_2$]Cl_2. 2H_2O [2] and [Cu (p-nbhtsc)$_2$]Cl_2.2H_2O [3], where (p-clbhtsc) = para-chloro benzaldehyde thiosemicarbazone , (p-mbhhtsc) = p-methoxy benzaldehyde thiosemicarbazone, (p-nbhtsc) = para-nitro benzaldehyde thiosemicarbazone; have been synthesized and characterized by elemental analysis, IR, H^1NMR, ^{13}CNMR, LC-MS, and electronic spectra. The magnetic moments and electronic spectral studies suggest distorted octahedral geometry for all Cu (II) complexes. The cytotoxicity activity against breast cancer cell lines MCF-7, antibacterial and antioxidant activities were evaluated for all the above compounds. The synthesized compounds were screened for their *in vitro* antibacterial activity using Disc Diffusion method against various strains of gram negative and gram positive bacteria. Tetracyclin was used as standard drug in the test. All these macrocyclic Cu (II) complexes evaluate their superoxide dismutase activity to scavenge the superoxide anions generated by the DPPH and ferric oxide oxidase system.

KEYWORDS: Copper (II) complexes, Thiosemicarbazide, macrocyclic, cytotoxicity, tumor cell lines, antibacterial.

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INTRODUCTION

The success of cis-platin has stimulated the development of metal based compounds[1-4]. Cancer is undoubtedly one of the main health concerns facing our society and one of the primary targets regarding medicinal chemistry. Even though platinum-based complexes have been in primary focus of research on chemotherapy agents[5-7], the interest in this field has shifted to non-platinum based agents[8-14], In order to find different metal complexes with less side effects and similar or better cytotoxicity. Thus, a wide variety of metal complexes based on titanium, gallium, germanium, palladium, gold, cobalt, ruthenium and tin are being intensively studied as platinum replacements. Furthermore, copper (II) based complexes appear to be very promising candidates for anticancer therapy; an idea supported by a considerable number of research articles describing the synthesis and cytotoxic activities of numerous copper (II) complexes[15-18]. Schiff base macrocyclic ligands based on thiosemicarbazone and their complexes have received considerable attention, because of their pharmacological properties they have numerous applications, for example as antibacterial and anticancer agents[19-21]. They can yield mono or polynuclear complexes, some of which are biologically relevant[22-25]. For example, some copper complexes can serve as models for enzymes such as galactose oxidase and may be used as effective oxidant and redox catalysts[26-27]. Furthermore, they allow selective complex and extraction of metallic cations and anions of biochemical and environmental importance[28-31]. Macrocyclic complexes are of great importance in enhancing various industrial applications and in a number of biological processes such as photosynthesis and dioxygen transport[32] , catalytic properties, potential applications as metal extractants, radiotherapeutic and medical imaging agents and potency towards DNA binding[33] with a high potential in antitumor therapy. For many years, it has been known that a large number of bis-thiosemicarbazones and their copper complexes showed promising antitumor activities[34-36]. A critical property of many of copper (II) complexes is the poor water solubility and the relatively high *in vivo* toxicity[37-38]. Many attempts have been made in the last decades to improve the hydrophilicity and reduce the toxic effects by modifying the thiosemicarbazones frameworks by the copper complexes[39]. In recent years, several families of copper complexes have been studied as potential antitumor agents. Although only a little understanding of the molecular basis of their mechanism of action has been documented, copper complexes have attracted attention based on modes of action different from that of cisplatin (covalent binding to DNA). Therefore, copper complexes may provide, at least in principle, a broader spectrum of antitumor activity. Synthetic superoxide dismutase mimetics have emerged as a potential novel class of drugs for the treatment of oxidative stress related disease. Among these agents metal complexes with macrocyclic ligands constitute an important group.As a part of our work involving the preparation of free imino-macrocyclic compounds, and their copper complexes, we are interested in obtaining the free [2+2] condensation product from carbohydrazone and thiosemicarbazide. The aim of our work was to compare the reactivity of different macrocyclic ligands containing the different functional group, but with variable numbers and different structures, with a copper metal ion. In addition, we determined the best conditions to control the nature of the copper complexes, since the different structures can improve their potential applications. Therefore, we report the anticancer, antibacterial and antioxidant evaluation of macrocyclic copper (II) complexes derived from thiosemicarbazide.

1. Experimental

1.1 Chemicals

All the chemicals used in the study are of analytical grade unless reported. The organic chemicals viz. p-methoxy benzaldehyde, p-chloro benzaldehyde, p-nitro benzaldehyde were purchased from merck pvt. Ltd.

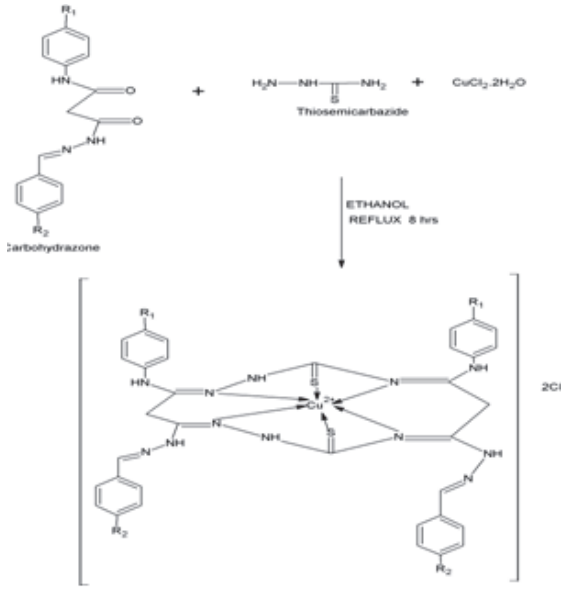
Thiosemicarbazide was obtained from S D Fine chemicals; copper (II) chloride and other organic solvents were purchased from the commercial sources and are used without further purification.

1.2 Apparatus

All glasswares were dried in an open flame before use in connection with an inert atmosphere. Solvents were evaporated under reduced pressure and evaporation was carried out at temperature <50 °C. Thin layer Chromatography was performed using silica gel 60 F_{254} plates with detecting agents iodine vapors spraying with 5% Sulphuric acid in ethanol followed by heating at 100°C, Tetra methyl silane (0.0 ppm) was used as an internal standard in ^{1}HNMR and $CDCl_3$ (77.0 ppm) was used in ^{13}CNMR. The abbreviations used to indicate the peak multiplicity were; S, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; Hz, Hertz. FAB MS was recorded on Jeol (Japan) / SX-102. Infrared spectrum was taken with KBr on Perkin – Elmer RX-1. Melting points were determined on a Buchi 535 digital melting point apparatus and were uncorrected. Elemental analysis was performed on a Perkin –Elmer 2400 C, H, N analyzer and values were within ± 0.5% of the calculated values. Anhydrous sodium sulphate (Na_2SO_4) was used as drying agent for the organic phases containing the compounds. unless otherwise stated.

1.3 Template synthesis of macrocyclic copper (II) complexes

A series of three macrocyclic Cu (II) complexes were synthesized by following procedure previously described[40]. To a mixture of the appropriate hydrated copper chloride (1 mmol) in absolute ethanol (10 ml) and Carbohydrazone (2 mmol) in absolute ethanol (20 ml) and thiosemicarbazide (2 mmol) in absolute ethanol (15 ml) was added slowly with strring. After the addition of thiosemicarbazide, 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 (scheme-1).



Where R_1 = alkyl group and R_2 = - Cl, -NO_3 and -OCH_3 groups

Scheme 1 Synthesis of macrocyclic copper (II) complexes.

2.3(a) Synthesis of bis (4-chlorobenzylidene thiosemicarbazone) copper (II) chloride.

Yield: 75 % m.p. 210 °C, color greenish yellow ;[Cu ($C_{37}H_{36}N_{12}Cl_2S_2$)]$Cl_2$. $2H_2O$, C = 54.0; H = 6.2; N = 24.2; S = 13.5%; Found: C = 54.2; H = 6.4 ; N = 24.4; S = 13.7 %; IR (KBr)(cm^{-1}) : 3199, 1591 : λ_{MAX} = 382 nm; ^{1}NMR (ppm): 2.45, 9.89, 8.35, 2.2 and 6.7; ^{13}CNMR : 20.43, 38.86-40.11, 55.33, 126.0-129.37, 136.33, 145.32; ESI – MS:777.36 a.m.u., molar conductivity = 7.7 $\Omega^{-1}cm^2mol^{-1}$.

2.3(b) Synthesis of bis (4-methoxy benzylidene thiosemicarbazone) copper (II) chloride.

Yield: 50 % m.p. 225°C, color green; [Cu ($C_{39}H_{42}N_{12}O_2S_2$)]Cl_2. $2H_2O$; C = 54.39; H = 6.28; N = 24.8; S = 14.2%; Found: C = 54.50; H = 6.32; N = 25; S = 14.6 %; IR (KBr) (cm^{-1}) : 3251, 2979, 1681, 1605, 1088 : λ_{MAX} = 380 nm; H^{1}NMR (ppm): 2.4, 9.99, 8.2, 2.1 and 6.8; ^{13}CNMR : 20.45, 39.01-40.26, 119.13, 128.22-129.32, 132.07-133.07; ESI – MS: 702.4(observed peak) other peak, 518.9, 380,366,352, 274, 153. a.m.u. molar conductivity = 12.5 $\Omega^{-1}cm^2mol^{-1}$.

2.3(c) Synthesis of bis (4- nitro benzylidene thiosemicarbazone) copper (II) chloride.

Yield: 80 % m.p. 240 °C, color yellow green; [Cu ($C_{37}H_{36}N_{14}O_4S_2$)]Cl_2 , C = 56.38; H = 5.86; N = 24.89; S = 15.6 %; Found: C = 56.40; H = 5.92; N = 24.92; S = 15.8 %; IR (KBr) (cm^{-1}): 3254, 1684, 1102, 1604 : λ_{MAX} = 280 nm; H^{1}NMR (ppm):3.1-3.07, 2.52, 8.6-8.7, 2.25, 7.0-8.3; ^{13}CNMR : 20.42, 38.88, 40.14, 118.96, 123.78-147.83; ESI – MS: 599.1 (molecular ion peak) other peak 405, 363, 272, 153.a.m.u. molar conductivity = 10.2 $\Omega^{-1}cm^2mol^{-1}$.

1.4 In-Vitro antitumor activity

Cell viability was assessed by the MTT staining method[41].In vitro cytotoxicity assays on cultured human tumor cell line still represent the standard method for the initial screening of antitumor agents. Thus as a first step to assess their pharmacological properties, the synthesized copper (II) complexes were assayed against the human breast tumor MCF-7 cell line. The cells were routinely maintained at 37°C in a humidified 5% CO_2 atmosphere with Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). After reaching confluence, the cells were detached by tripsinization and counted. For the cytotoxicity assay, 4 $\times 10^4$ cells well^{-1} were seeded in 100 µL of complete medium in 96-well plates (corning Costar). The plates were incubated at 37°C in 5% CO_2 for 24 hrs to allow cell adhesion prior to drug testing. The complex was dissolved in sterile dimethylsulfoxide (dmso, stock solution with maximum concentration of 20 mmol L^{-1}) and dilute to 0.5µM, 1µM, 2µM, 5 µM, 10 µM and 15µM. Two microliters of each complex sample were added to 100 µL medium (without FBS). In the control experiments, cells were grown in the same media without the compounds. Relative cell viability was evaluated by measuring the optical density at 570 nm on microplate reader (Quant Bio-tek Instruments, Inc.). At 20 µM concentration of the synthesized compounds, three different experiments were carried in triplicates and reported as the cell cytotoxicity for each compound.

1.5 Antibacterial activity

Antibacterial activities of the complexes were tested against using muller Hinton agar medium[42-43]. 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 hr. at room temperature and incubated at 37 °C for 24 hrs[44].

1.6 Antioxidant activity

1.72.6(a) DPPH activity

The free radical scavenging activity (RSA) of compounds 1-3 at concentration 200, 400, 800 µg/ml was carried out in presence of freshly prepared solution of stable free radical DPPH (0.04% w/v) following Hataro's method[45] using ascorbic acid as standard. All the test analysis was performed on three triplicates and results are averaged. The results in percentage are expressed as the ratio of absorption decrease

of DPPH in the presence of test compounds and absorption of DPPH in the absence of test compounds at 517 nm by UV Visible spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using following equation-

% RSA = (AC-AS) ×100/AS

Where, AC = Absorbance of control.

AS = Absorbance of test sample

2.6(b) Reducing power

The reducing power of the synthesized compound 1-3 was determined according to the literature method[46] at defferent concentration of samples (200, 400 and 800 µg/ml) in DMSO (1 ml) were mixed with phosphate buffer (2.5 ml, 0.02 M, pH=6.6) and potassium ferricyanide at 50 °C for 20 min, and after which a portion (2.5 ml of trichloro acetic acid (10%) was added to the mixture, centrifuged for 10 min. at 1000 ×g. The upper layer of solution (25 ml) mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1 %) and the absorbance at 700 nm was measured in a spectrophotometer. The higher absorbance of the reaction mixture indicated greater reducing power.

RESULTS AND DISCUSSION

1.8 Infrared spectra

The Infrared spectrum of the synthesized compounds was often used as the tool to establish the structure in the absence of the single crystal analysis. The infrared spectroscopic data obtained for the respective copper (II) complexes were found to be in good agreement with the structural results provided by other spectroscopic techniques. The main IR vibration bands of the macrocyclic Cu(II) complexes are listed in table 1. Upon coordination , change in the v (C=S), v(C=N) and v (N-H) wave numbers, in comparison to the values found for the thiosemicarbazone were observed for complexes 1-3. They are consistent with the tridentate coordination of the thiosemicarbazone derivatives through the thiolate sulfur and azomethine nitrogen atoms[47]. The occurrence of the v (N-N) band at higher frequencies in the IR spectra of the complexes compared to those observed for the ligands, confirms coordination through the azomethine nitrogen atom[48]. The v (C=S) bands at 801-860 cm^{-1} in the spectra of carbohydrazone ligand shift to the (782-786) cm^{-1} range in the complexes, indicating coordination through the sulfur atom. These shifts to lower frequencies are compatible with deprotonation and formation of a C-S single bond[49].

Table 1
Relevant IR spectral assignments (cm^{-1}) of synthesized macrocyclic Cu(II) complexes.

S.No	Compounds	v (-NH)	v (Ar-H)	v (-C=S)	v (-C=N)
1.	$[Cu\ (C_{37}H_{36}N_{12}Cl_2S_2)]Cl_2.2H_2O$	3199	2978	1084	1591
2.	$[Cu\ (C_{39}H_{42}N_{12}O_2S_2)]Cl_2.2H_2O$	3251	2979	1088	1605
3.	$[Cu\ (C_{37}H_{36}N_{14}O_4S_2)]Cl_2.2H_2O$	3254	2989	1102	1604

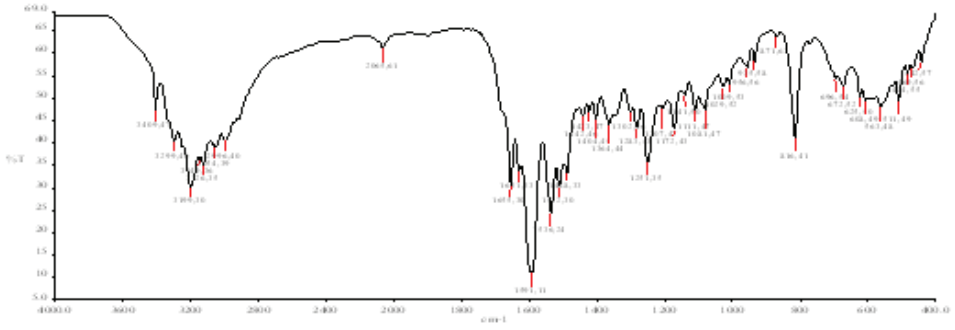


Figure 1
IR spectra of [Cu ($C_{37}H_{36}N_{12}Cl_2S_2$)] $Cl_2.2H_2O$ complex.

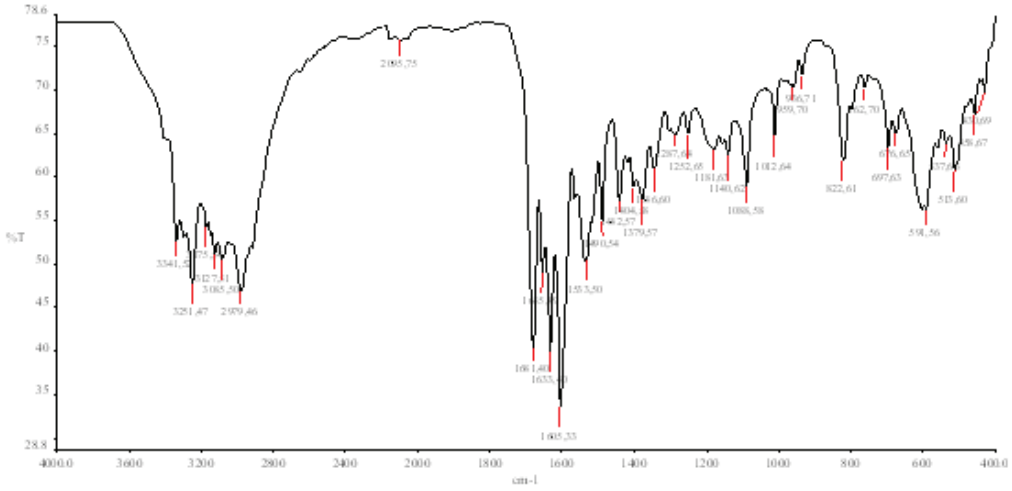


Figure 2
IR spectra of Spectra [Cu ($C_{39}H_{42}N_{12}O_2S_2$)]$Cl_2.2H_2O$ complex.

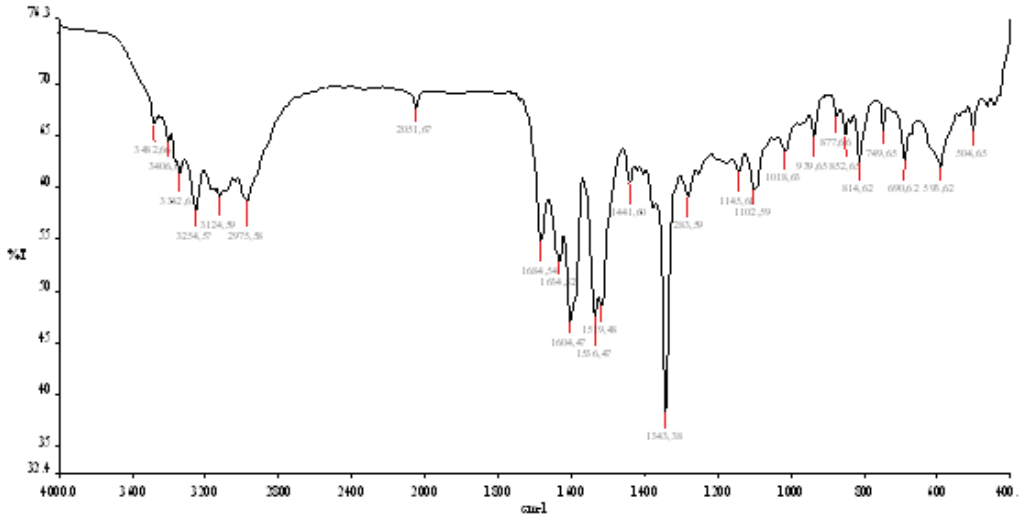


Figure 3
IR spectra of [Cu ($C_{37}H_{36}N_{14}O_4S_2$)]Cl_2.2H_2O complex.

1.9 ^{1}HNMR 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 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^{1}NMR spectra of macrocyclic Cu (II) complexes shows signals corresponding to –CH_3, -NH_2, NH (hydrazone) and at 2.28 (5, 3H), 7.40-7.48 (M, 3H), 8.059-8.38 (2H) and 10.09 (s, 1H). The NMR spectrum of metal chelates confirms the participation of –NH_2 group and imino –NH group in the coordination with metal ions. The three new Cu (II) complexes show similar ^{1}H – Chemical shift behavior. Some hydrogen atom values of δ were not observed precisely due to overlapping with the signals of the aromatic hydrogen atoms of ligand. ^{1}HNMR integrations and signal multiplicity are in agreement with the proposed structures. In the ^{1}HNMR spectra of the complexes 1, 2, and 3, a high frequency shift of Ca 0.13 ppm, for the methyl hydrogen atoms (C-CH_3), compared to the spectra of the carbohydrazone ligand, evidences the coordinator through the azomethine nitrogen atom.

Table 2
Relevant proton NMR spectral assignments (ppm) of synthesized macrocyclic Cu (II) complexes

S.No.	Compounds	δ (-NH)	δ (CH_2)	δ (HC=N)	δ (CH_3)	δ Ar (C-H)
1.	[Cu ($C_{37}H_{36}N_{12}Cl_2S_2$)]$Cl_2$.2$H_2O$	3.42-3.45	2.35	8.3-8.5	2.02	6.81-7.31
2.	[Cu ($C_{39}H_{42}N_{12}O_2S_2$)]Cl_2.2H_2O	3.05-3.5	2.1	8.5-8.7	1.09	6.99-7.9
3.	[Cu ($C_{37}H_{36}N_{14}O_4S_2$)]Cl_2.2H_2O	3.1-3.07	2.52	8.6-8.7	2.25-2.5	7.0-8.3

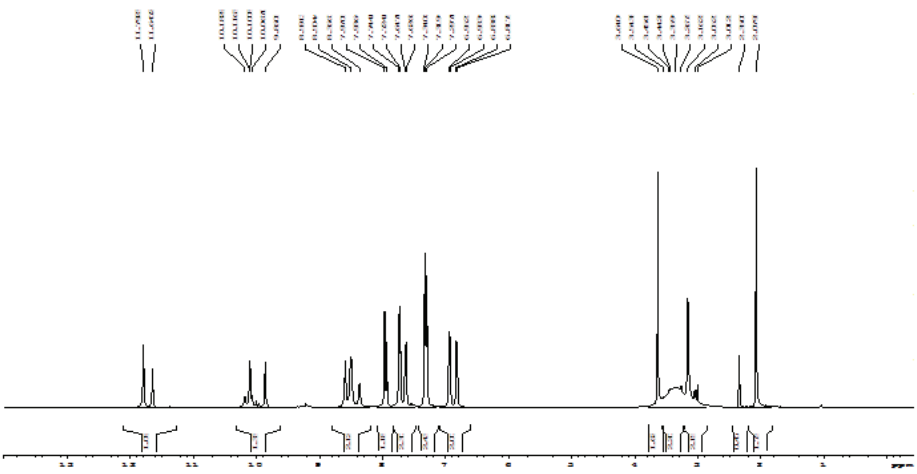


Figure 4
^{1}HNMR spectra of [Cu ($C_{37}H_{36}N_{12}Cl_2S_2$)]$Cl_2$.2$H_2O$ complexe.

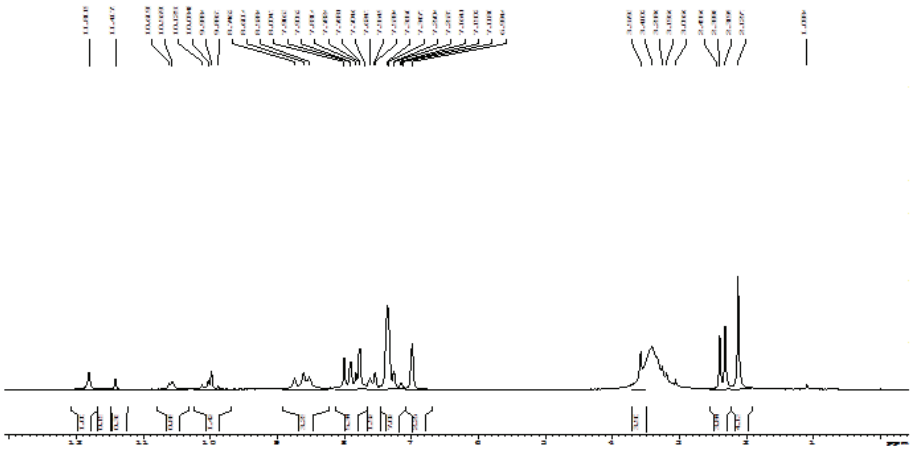


Figure 5
***^{1}HNMR spectra of [Cu ($C_{39}H_{42}N_{12}O_2S_2$)]Cl_2.2H_2O complex*.**

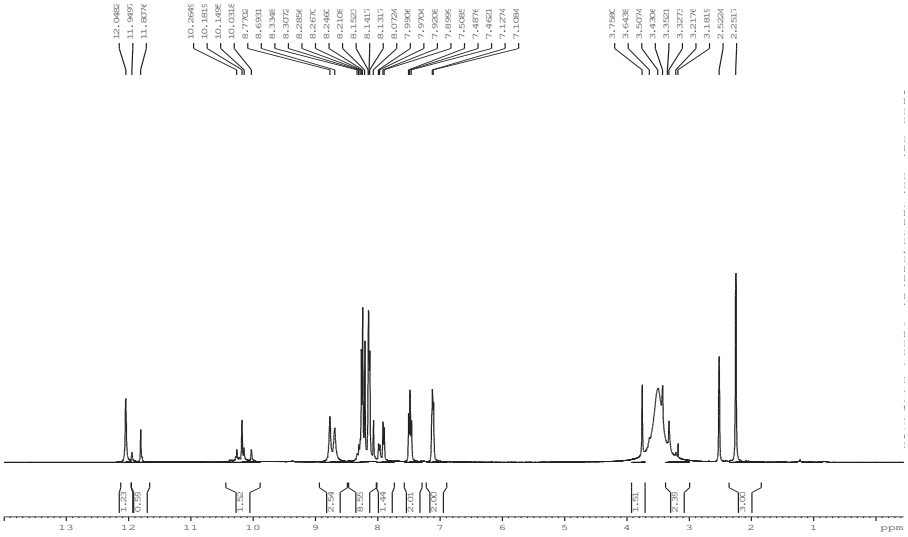


Figure 6
^{1}HNMR spectra of [Cu ($C_{37}H_{36}N_{14}O_4S_2$)]Cl_2.2H_2O complex.

1.10 ^{13}CNMR spectra

In the ^{13}CNMR spectrum of synthesized macrocyclic Cu(II) complexes indicated new resonances are 20.43, 20.45, 20.42 (-CH_3), 126-129.37, 128.22-129.32, 123.78-147.83 (Ar-C), 119.06, 119.13, 118.9 (C=N)and 38.86-40.11, 39.01-40.26, 38.88 correspondence to respective complexes 1,2 and 3 complexes.

Table 3
Relevant ^{13}CNMR spectra assignments (ppm) of synthesized macrocyclic Cu (II) complexes.

S.No.	Compounds	δ (-CH_3)	δ (Ar-C)	δ (C=N)	δ (CH_2)
1.	[Cu ($C_{37}H_{36}N_{12}Cl_2S_2$)]$Cl_2$.2$H_2O$	20.43	126-129.37	119.06	38.86-40.11
2.	[Cu ($C_{39}H_{42}N_{12}O_2S_2$)]Cl_2.2H_2O	20.45	128.22-129.32	119.13	39.01-40.26
3.	[Cu ($C_{37}H_{36}N_{14}O_4S_2$)]Cl_2.2H_2O	20.42	123.78-147.83	118.9	38.88

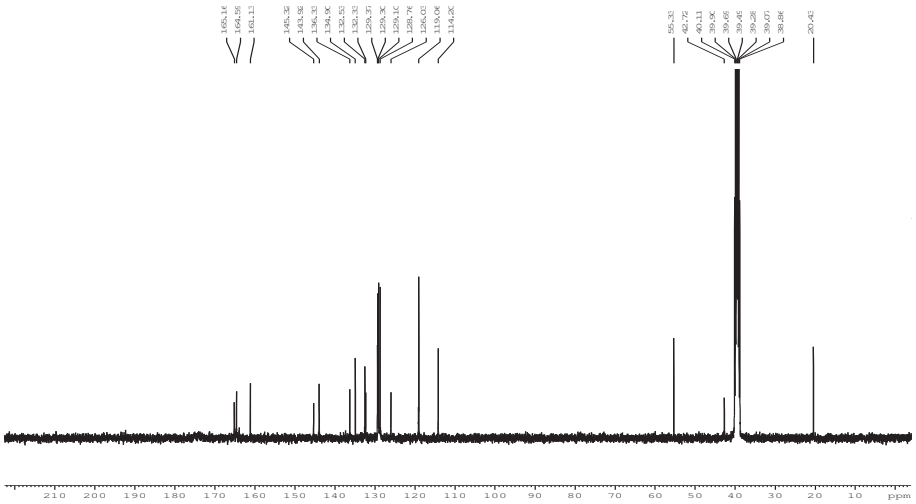


Figure 7
^{13}CNMR spectra of [Cu ($C_{37}H_{36}N_{12}Cl_2S_2$)]$Cl_2$.2$H_2O$ complex.

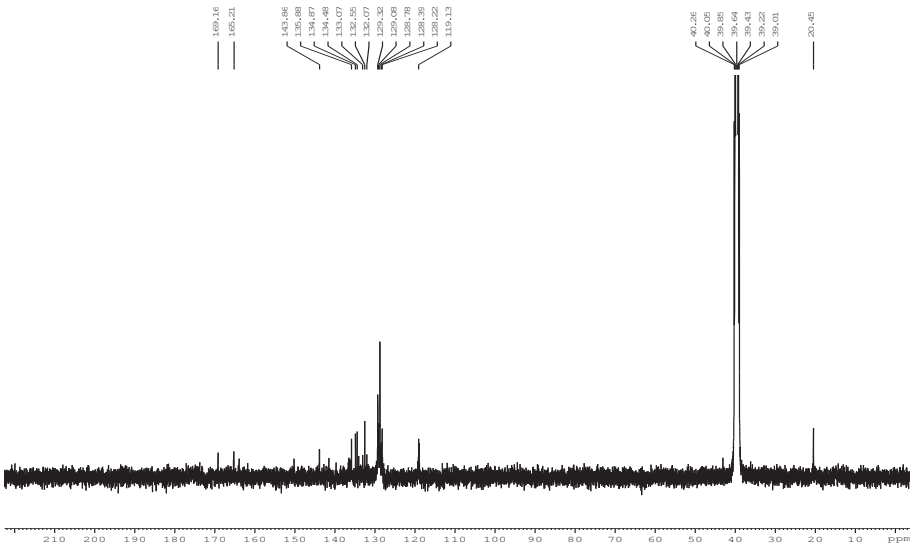


Figure 8
^{13}CNMR spectra of [Cu ($C_{39}H_{42}N_{12}O_2S_2$)]Cl_2.2H_2O complex.

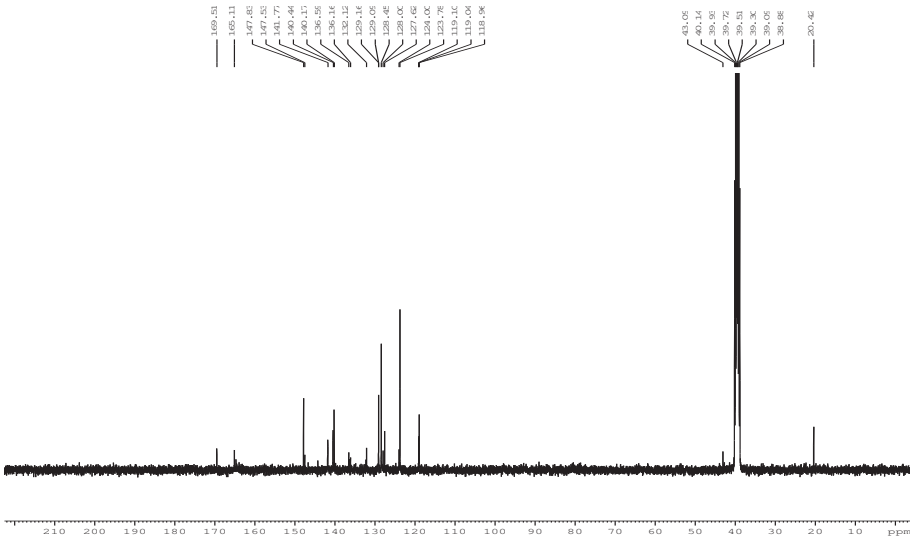
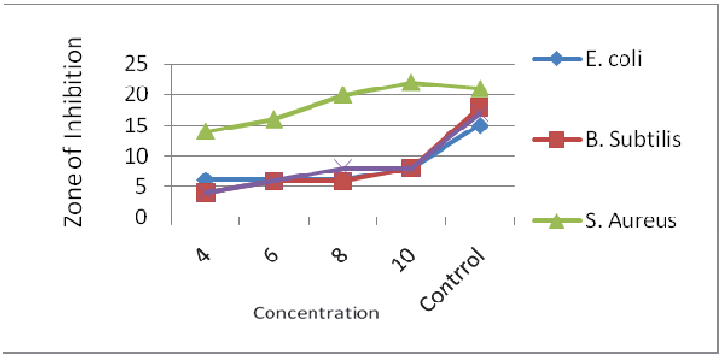


Figure 9
^{13}CNMR spectra of [Cu ($C_{37}H_{36}N_{14}O_4S_2$)]Cl_2.2H_2O complex.

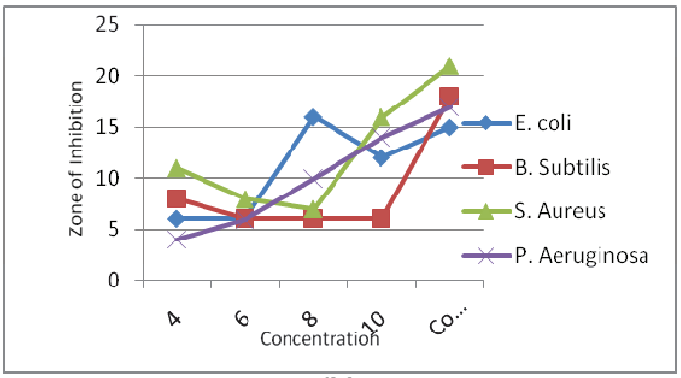
1.11 Antibacterial activity

Three chemically synthesized Cu (II) macrocyclic complexes were tested in-vitro for their antibacterial activity against four test bacteria namely E. Coli, S. Aureus, B. Subtilis and P.Aureogenosa. The minimum inhibitory concentrations of complexes were determined by disc diffusion method. The minimum inhibitory concentration at which no growth observed was taken as the MIC values. All the complexes of the tested series possessed good antibacterial activity against both gram-positive and gram-negative bacteria. The higher antibacterial activity of the copper (II) complexes may be due to coordination and chelating tends to make metal complexes act as more powerful and potent bactereostatic agents, thus inhibiting the growth of the bacteria. The three copper (II) complexes were also compared with commercial antibiotic

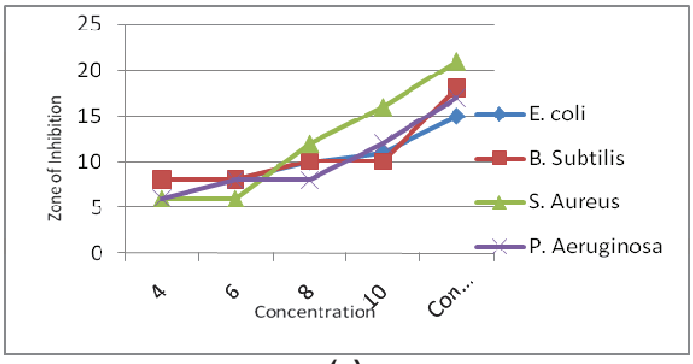
namely tetracycline. All these copper (II) complexes are active due to the presence of thio group in the coordinated ligand. Comparison of zone of inhibition of Cu (II) macrocyclic complexes against concentration and standard drug against different bacteria are given in bar graph.



(a)



(b)



(c)

1.12 Anti-oxidant activity

All the newly synthesized compounds were tested for their radical scavenging activity (RSA) using stable free radical DPPH. The results were compared with the standard ascorbic acid.

Table 4
Antioxidant activity of synthesized macrocyclic Cu(II) complexes.

Compounds	Radical scavenging activity(RSA) in µg/ml			IC_{50} µg/ml	Reducing power concentration µg/ml		
	200	400	800		200	400	800
[Cu ($C_{37}H_{36}N_{12}Cl_2S_2$)]$Cl_2$.2$H_2O$	24.29	63.82	68.36	40.61	0.098	0.0108	0.132
[Cu ($C_{39}H_{42}N_{12}O_2S_2$)]Cl_2.2H_2O	70.05	67.51	72.59	20.66	0.189	0.198	0.216
[Cu ($C_{37}H_{36}N_{14}O_4S_2$)]Cl_2.2H_2O	43.22	68.06	71.42	29.62	0.263	0.299	0.334
Ascorbic Acid	82.03	76.04	85.32	25.05	0.236	0.312	0.368

Compound – 2 exhibited 70.05 % radical scavenging activity at concentration 200 µg/ml, compounds 1, 2 and three showed 63.82, 67.51 and 68.06 % radical scavenging activity at concentration 400 µg/ml where as compounds 1, 2 and 3rd exhibited 68.36, 72.59 and 71.42 radical scavenging activity at concentration 800 µg/ml respectively. The TSA of all compounds may be due to the presence of NH groups which may donate an electron or hydrogen atom to DPPH and form a stable free radical. This radical can be stabilized by delocalization. This fact confirm their good electron and /or hydrogen donating ability and to act as a radical scavenger. However, none of the compounds exhibited radical scavenging activity betters than standard ascorbic acid.

1.13 Cytotoxic activity

All macrocyclic copper (II) complexes were evaluated for their effectiveness against the breast tumor cell line MCF-7, for comparison purpose, the cytotoxicity of cis-platin was evaluated under the same experimental conditions. The values of cell viability were calculated after the tested compounds were incubated for 48 hrs. The IC_{50} values, calculated from the close survival curves from MTT assay. Which are shown in table - 5. Comparing only the values of IC_{50} of all macrocyclic copper (II) complexes, the order of cytotoxic activity was [Cu ($C_{37}H_{36}N_{12}Cl_2S_2$)]$Cl_2$. 2$H_2O$ > [Cu ($C_{39}H_{42}N_{12}O_2S_2$)]Cl_2.2H_2O > [Cu ($C_{37}H_{36}N_{14}O_4S_2$)]Cl_2.2H_2O suggesting that the activity is increased by the presence of bulky groups bonded to $N_{(4)}$ of the thiosemicarbazone macrocyclic ligand. The good values of activity found for these complexes, around 5.0 µmol^{-1}, show that the complexation of macrocyclic thiosemicarbazone to Cu(II) may be a good strategy to obtain antitumor agents . The similarity of the values of IC_{50} found for the Cu (II) complexes is evidence in favour of the same biochemical action mechanism but the different from those of the cis-platin inactive in this case. In fact, the literature reports that Cu (II) complexes of macrocyclic thiosemicarbazone derivatives are able to bind to DNA in-vitro and present enhanced capacity to form interstrand crosslinks when compared to cis-platin[50-51]. All copper (II) complexes could present antitumor effect by inhibiting DNA synthesis through the blockage of the enzyme ribonucleoside diphospho reductase (RDR), which catalyses the conversion of ribonucleotides into deoxyribonucleotides, as proposed for other α (N) – heterocyclic thiosemicarbazone[52].

Table 5
IC_{50} (µm) values of synthesized macrocyclic copper (II) complexes.

S. No.	Compound	R_1	R_2	LD_{50} value
1.	[Cu ($C_{37}H_{36}N_{12}Cl_2S_2$)]$Cl_2$.2$H_2O$	$-CH_3$	-Cl	10 µM
2.	[Cu ($C_{39}H_{42}N_{12}O_2S_2$)]Cl_2.2H_2O	$-CH_3$	$-OCH_3$	15 µM
3.	[Cu ($C_{37}H_{36}N_{14}O_4S_2$)]Cl_2.2H_2O	$-CH_3$	$-NO_3$	inactive

Morphological Changes

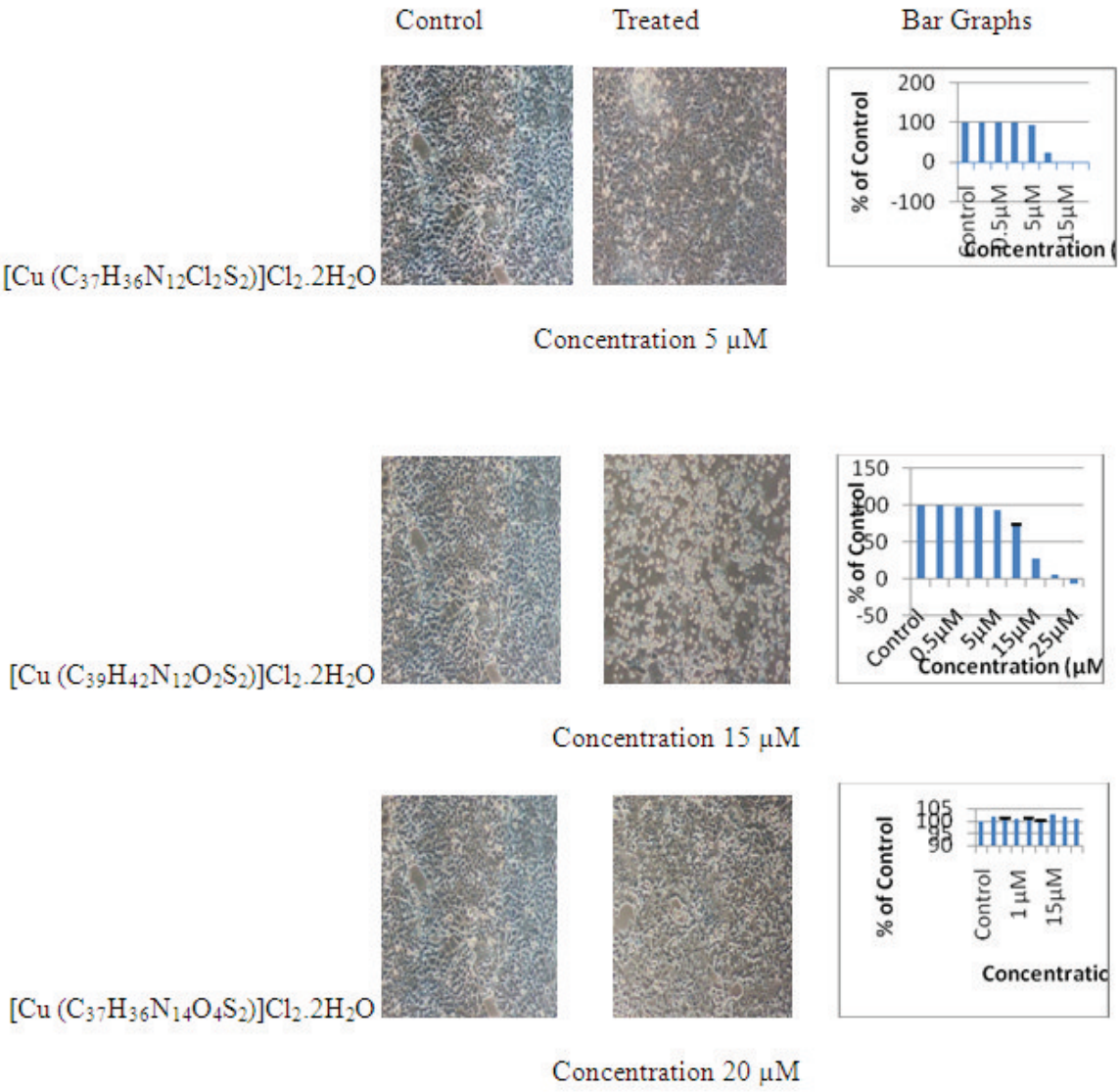


Figure.10
Cells treated with or without 20 µM of 1-3 complexes for 24 hrs. Morphological changes indicate that complexes induced cell death is mostly via apoptosis.

CONCLUSION

This article presents a survey of synthesis, structure and antitumor evaluation of the macrocyclic copper (II) complexes. All Cu (II) macrocyclic complexes were synthesized and well characterized in detail by FTIR, ^{1}HNMR, ^{13}CNMR analysis. All Cu (II) complexes are in a distorted octahedral environment with the ligand having a tetradentate (C, N) chelating motif. All three Cu (II) complexes show modest invitro cytotoxic properties against breast cancer cell line MCF-7. IC_{50} values are compared with cisplatin and the results revealed that complex 1 possesses better activity than 2 and 3. More detailed studies are needed to understand the

mechanisms of action at the cellular level and the role of the metal. Investigation of antibacterial screening data revealed that the compounds 1, 2 and 3 exhibited maximum zone of inhibition against the bacterial strains E. coli, S. Aureus, B. Subtilis and P. Aerogeunosa. Analysis of result revealed that the all macrocyclic Cu (II) complexes exhibited good radical scavenging activity as compared to the standard ascorbic acid. Compounds 3 and 4 may reduce metal ions complexes to their lower oxidation state or take part in electron transfer reaction. These compounds showed the ability of electron donor to scavenge free radicals and rest of the compounds showed lower absorbance as compared to the standard ascorbic acid. Apparently, potency of complex 3 was 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 blebbling, 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 lines were treated with the macrocyclic Cu (II) complexes (10 hrs) for more potent 24 hrs. The data reported in this article may be helpful guide for the medicinal chemist who is working in this area.

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