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Mononuclear copper (II) macrocyclic complexes derived from malonanilic carbohydrazone and thiosemicarbazide: Synthesis, spectral characterization and biological evaluation

Brajraj Sharma[a], Rumana Ahmad[b†*], Richa Kothari[a], Jyoti Kushwaha[b], Deepti Mukhraiya[b] and Anil K. Balapure[c]

[a]*School of Science, Department of Chemistry, ITM University, Gwalior, MP, India*
[b]*School of Life Sciences, Department of Biotechnology, ITM University, Gwalior, MP, India*
[c]*Tissue & Cell Culture Unit (TCCU), replaced with (Division of Biochemistry), CSIR-Central Drug Research Institute, Lucknow, UP, India*
[†]*Present Affiliation: Department of Biochemistry, Era's Lucknow Medical College & Hospital Sarfarazganj, Hardoi Road, Lucknow, UP, India*

ABSTRACT

A series of six Cu (II) complexes were prepared from N^4 substituted thiosemicarbazones having structures [Cu (p-clbhtsc)$_2$]Cl$_2$. 2H$_2$O [1], [Cu (p-mbhtsc)$_2$]Cl$_2$.2H$_2$O [2], [Cu (p-nbhtsc)$_2$]Cl$_2$.2H$_2$O [3], [Cu (p-clacehtsc)$_2$]Cl$_2$. 2H$_2$O [4], [Cu (p-macehtsc)$_2$]Cl$_2$.2H$_2$O [5] and [Cu (p-nacehtsc)$_2$]Cl$_2$.2H$_2$O [6] where (p-clbhtsc)=p-chlorobenzylidene thiosemicarbazone, (p-mbhtsc)=p-methoxy benzylidene thiosemicarbazone, (p-nbhtsc)=p-nitro benzylidene thiosemicarbazone, (p-clacehtsc)=p-chloroacetophenone thiosemicarbazone, (p-macehtsc)=p-methoxyacetophenone thiosemicarbazone, and (p-nacehtsc)=p-nitroacetophenone thiosemicarbazone. All six complexes were characterized by elemental analysis, IR, ^{1}HNMR, mass and electronic spectra. The magnetic moments and electronic spectral studies suggested distorted octahedral geometry for all the complexes. The monoanionic thiosemicarbazonate ligands act in a tridentate mode, binding through azomethine nitrogen and sulfur atom. Cytotoxic activity against human breast cancer cell line MCF-7, antibacterial and antioxidant activities were evaluated for all the six complexes. Out of the six Cu (II) complexes, five showed significant activity against the studied cell line; having IC$_{50}$ values in the range 2-12 µM. The standard antibreast cancer drug Tamoxifen was used as a positive control. The synthesized compounds were screened for their in vitro antibacterial activity using Disc Diffusion method against two strains each of gram negative and gram positive bacteria. Tetracycline was used as positive control in the test. All the compounds showed significant antibacterial activity in the range of 2-10 mg/ml. Antioxidant activity of the six macrocyclic copper complexes was screened using the H$_2$O$_2$ scavenging assay. All six complexes exhibited potent antioxidant activity in the range 35-58%. The compounds would be evaluated further for their possible DNA binding, cleavage, antifungal and anti-diabetic activities.

Keywords: Copper (II) complexes, thiosemicarbazide, tetra-azamacrocycles, cytotoxicity, anticancer, antibacterial, antioxidant activity.

INTRODUCTION

The success of cisplatin has stimulated the development of metal based compounds [1-4]. Cancer is undoubtedly one of the main health concerns and primary targets for Medicinal Chemistry. Even though platinum-based complexes have been in primary focus as chemotherapeutic 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 lesser 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 extensively studied as platinum replacements. Copper (II) based complexes appear to be very promising candidates for anticancer therapy; as evidenced 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 its complexes have received considerable attention. Because of their pharmacological properties, they have numerous applications as antibacterial and anticancer agents [19-21]. They can yield mono or polynuclear complexes, some of which are biologically relevant [22-25]. Copper complexes can serve as models for enzymes such as galactose oxidase and may be used as effective oxidants and redox catalysts [26-27]. Furthermore, they allow extraction of metallic cations and anions of biochemical and environmental importance [28-31]. Macrocyclic ligand complexes find applications in various industries and in a number of biological processes such as photosynthesis and dioxygen transport [32], catalysis, metal extractants, radiotherapy, medical imaging agents, DNA binding [33] and antitumor agents. It has been known for many years that a large number of bis-thiosemicarbazones and series of their copper complexes have promising antitumor activities [34-36]. A critical property of many copper (II) complexes is their poor water solubility and their relatively high *in vivo* toxicity [37-38]. Many attempts have been made to improve hydrophilicity and reduce toxicity by modifying the thiosemicarbazone framework [39]. In recent years, several series of copper complexes have been studied as potential antitumor agents. Although scanty information is available of the molecular basis of their mechanism of action, copper complexes have attracted attention because their probable mode of action is different from that of cisplatin. Therefore, copper complexes may provide a broader spectrum of antitumor activity.

Drug resistance has become a growing problem in the treatment of infectious diseases caused by bacteria [40].The serious medical problem of bacterial and fungal resistance and the rapid rate at which it develops has led to increasing levels of resistance to classical antibiotics, [41-42], and the discovery and development of effective antibacterial drugs with novel mechanisms of action have, thus, become urgent tasks for research programs on infectious diseases [43].The importance of metal ions in biological systems is well established. One of the most interesting features of metal-coordinated systems is the concerted spatial arrangement of the ligands around the metal ion. Among metal ions of biological importance, the Cu (II) ion is involved in a large number of distorted complexes [44]. Over the past two decades, considerable attention has been paid to metal complexes of Schiff bases containing nitrogen and other donor atoms [45-46]. Bio-organometallic chemistry is dedicated to the study of metallic complexes and their biological applications [47], including the design of new drugs that are more effective than those already known. Thiosemicarbazones are well established as an important class of sulfur-donor Schiff base ligands and their metal-complexing ability is responsible for the remarkable biological activities observed for these compounds.

As part of our work involving the preparation of free imino-macrocyclic compounds and their metal complexes, we were interested in obtaining the free [2+2] condensation product from carbohydrazone and thiosemicarbazide. The aim of this work was to compare and assess the reactivity of different macroligands containing the same functional group, but with variable number of substituents and different structures, with a copper metal ion. In addition, we determined the optimal conditions to control the nature of the copper complexes, since the different structures can improve their potential applications. Therefore, in the present paper, we report the synthesis, structural characterization and biological evaluation of mononuclear Cu (II) macrocyclic complexes derived from malonanilic carbohydrazone and thiosemicarbazide. All complexes exhibited potent anticancer, antioxidant and antibacterial activities.

MATERIALS AND METHODS

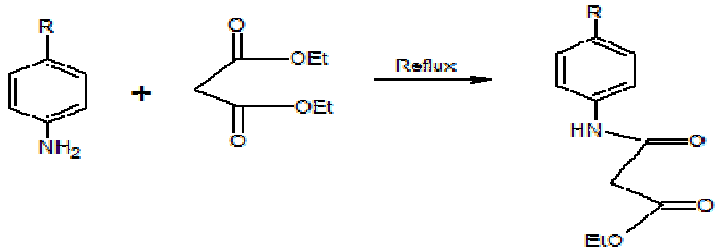
Chemistry

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 <50°C. TLC was performed using silica gel $60F_{254}$ plates with iodine vapors as detecting agent. Tetra methyl silane (TMS; 0.0 ppm) was used as an internal standard in ^{1}HNMR and Chloroform-d ($CDCl_3$; 77.0 ppm) was used in ^{13}C NMR. Elemental analysis was carried out on a Perkin Elmer 2400 series 11 CHNS/O elemental analyzer. FTIR spectra were recorded using KBr pellets on Perkin Elmer-Spectrum RX-IFTIR in the 4000-250 cm^{-1} region. The electronic spectra in DMSO solution were obtained with a Hitachi 330 uv-vis-nir spectrophotometer. ^{1}HNMR and ^{13}CNMR spectra were recorded on a FT-NMR Spectrometer model Avance-II (Bruker) using DMSO d6 as solvent and TMS as internal reference. The FAB Masses in positive mode were recorded on a Waters Micromass Q-Tof spectrometer; m-Nitro benzyl alcohol (m-NOBA) was used as the matrix. Melting points were determined by open capillary method. All materials were

obtained from commercial suppliers such as Merck, CDH, SRL and were used without further purification. The solvents and copper salts used were of analytical grade. Various hydrazones of complexation agent were prepared by standard methods described in the literature [48].

Synthesis of ethyl -2-(4-methoxyanilino) ethanoate (Scheme 1)
A mixture of p-toluidine and diethylmalonate (1:2) was refluxed for 30 minutes in a round bottom flask fitted with an 18" air condenser such that ethanol formed escaped and diethylmalonate collected in the flask. The contents were cooled, ethanol was added when malondianilide separated as solid. It was filtered under suction and filtrate containing ethylmalonianilate was slowly added with stirring on ice. The ester precipitated out as a white solid and was filtered, dried and re-crystallized from petroleum ether (Scheme 1).

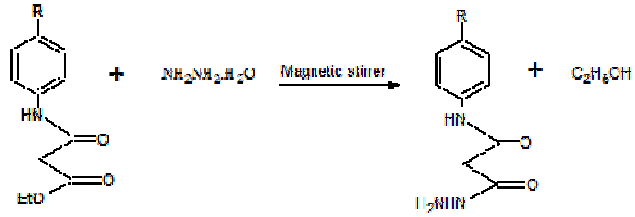
Yield: 45 %; mp 70-72 °C; IR (KBr) (cm^{-1}): 3345, 2983, 1726, 1685, 1537; H^1NMR: 1.27, 2.3, 7.0-7.4, 8.1 and 9.98; λ_{max} = 450 ESI MS M/Z: 222.2 a.m.u.



Scheme 1. Synthesis of ethyl-2-(4-methoxyanilino) ethanoate

Synthesis of malonanilic acid hydrazide
To a solution of ethylmalonanilate in ethanol, hydrazine hydrate was added. The mixture was stirred for about 10 minutes, when acid hydrazide separated and the contents were left overnight. The contents were filtered and re-crystallized twice from hot ethanol and acid hydrazide was obtained as white crystals (Scheme 2).

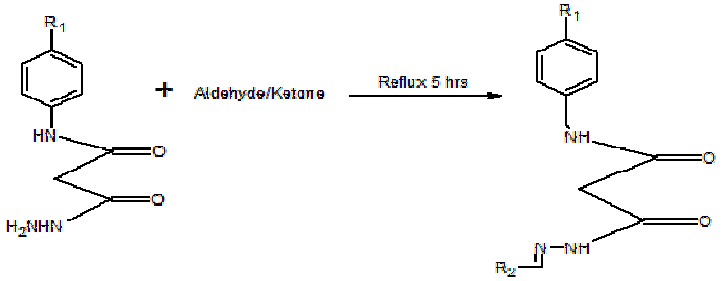
Yield: 45 %; mp 145°C; IR (KBr) (cm^{-1}): 3301, 3053, 1680, 1634, 1512; H^1NMR: 1.2, 2.2-2.5, 7.0-7.46, 8.2 and 9.94; λ_{max} = 410 ESI MS M/Z: 265 a.m.u.



Scheme 2. Synthesis of malonanilic acid hydrazide

Synthesis of malonanilic acid hydrazones
To a methanolic solution of acid hydrazide added the respective aldehyde/ketone and glacial acetic acid. The mixture was stirred for about 10 min and then refluxed for 5 h, and the contents were left overnight. The colored solid was filtered and re-crystallized twice from hot ethanol (Scheme 3).

Yield: 75-90% mp 220-260°C, IR (KBr) (cm^{-1}): 3285 (-NH-), 3073, 2960, 1686, 1652, 1606, and 1440 (m); H^1NMR: 2.2, 2.52-2.54, 3.7-3.8, 6.8-6.9, 7.4-7.9, 10.0 and 11.33; λ_{max} = 340 ESI MS M/Z: 326.2 a.m.u.

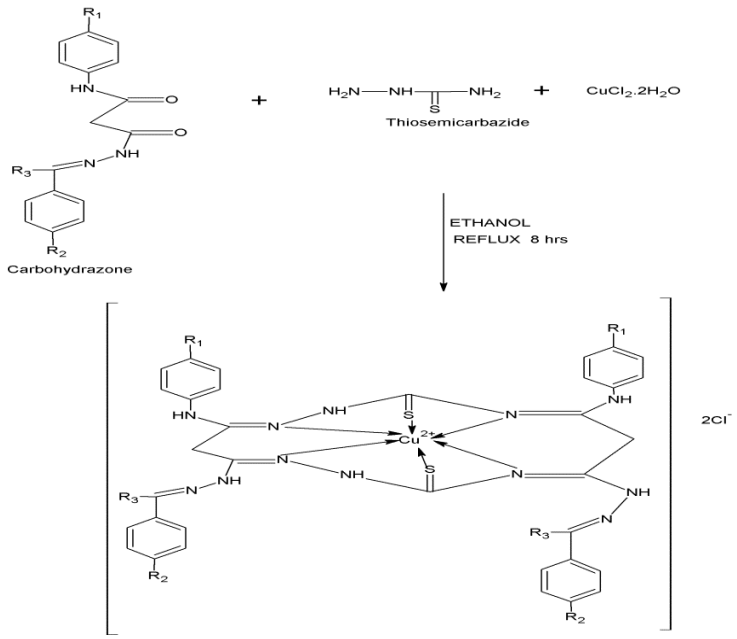


Scheme 3. Synthesis of malonanilic acid hydrazone

Where R_1 = CH_3 and R_2 = p-chloro benzaldehyde, p-methoxy benzaldehyde, p-nitrobenzaldehyde, p-chloroacetophenone, p-methoxy acetophenone and p-nitroacetophenone

Synthesis of macrocyclic Cu (II) complexes (1-6)

All these complexes were synthesized according to the method published previously [47]. Briefly, a mixture of the appropriate hydrated copper chloride in absolute ethanol, substituted carbohydrazone in absolute ethanol and thiosemicarbazide in absolute ethanol were added slowly with stirring. After the addition of thiosemicarbazide, the reaction was carried out for 8h 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 4).



Scheme 4: Synthesis of macrocyclic copper (II) complexes *via* template method

1: R_1 =CH_3, R_2 = Cl and R_3=H
2: R_1 =CH_3, R_2 = OCH_3 and R_3=H
3: R_1 =CH_3, R_2 = NO_3 and R_3=H
4: R_1 =CH_3, R_2 =Cl and R_3=CH_3
5: R_1 =CH_3, R_2 = OCH_3 and R_3=CH_3
6: R_1 =CH_3, R_2 = NO_3 and R_3=CH_3

Synthesis of macrocyclic copper (II) complexes via Template method

[Cu (p-clbhtsc)$_2$] Cl_2; bis (p-chlorobenzylidene thiosemicarbazone) copper II chloride (1)

Yield: 75 %; mp 210 °C; IR (KBr) (cm^{-1}): 3199, 1591; ^{1}H NMR (TMS) (ppm): 2.45, 9.89, 8.35, 2.2 and 6.7; ^{13}C NMR ($CDCl_3$): 20.43, 38.86-40.11, 55.33, 126.0-129.37, 136.33, 145.32; ESI MS m/z: 735 a.m.u.; Anal. Calcd for [Cu ($C_{37}H_{36}N_{12}S_2Cl_2$)]$Cl_2$.$2H_2O$; 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 %; μ_{eff} = 1.73-1.75 BM.

[Cu (pmbhtsc)$_2$]Cl_2; bis (p-methoxybenzylidene thiosemicarbazone) copper II chloride (2)

Yield: 50 %; mp 225°C; IR (KBr) (cm^{-1}): 3251, 2979, 1681, 1605, 1088; H^1 NMR (TMR) (ppm): 2.4, 9.99, 8.2, 2.1 and 6.8; ^{13}CNMR ($CDCl_3$): 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.; Anal. Calcd for [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 %; μ_{eff} = 1.73-1.76 BM.

[Cu (p-nbhtsc)$_2$]Cl$_2$; bis (p-nitrobenzylidene thiosemicarbazone) copper II chloride (3)
Yield: 80 %; mp 240 °C; IR (KBr) (cm^{-1}): 3254, 1684, 1102, 1604; H^1 NMR (TMS) (ppm):3.1-3.07, 2.52, 8.6-8.7, 2.25, 7.0-8.3; ^{13}C NMR ($CDCl_3$): 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; Anal. Calcd for [Cu ($C_{37}H_{36}N_{14}S_2O_4$)]Cl_2.2H_2O; 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 %; μ_{eff} = 1.73-1.76 BM.

[Cu (p-clacehtsc)$_2$]Cl$_2$; bis (p-chloroacetophenone thiosemicarbazone) copper II chloride (4)
Yield: 75 %; mp 250 °C; IR (KBr) (cm^{-1}): 3341, 2985, 1683, 1604, 33, 1094; H^1 NMR (TMS) (ppm): 1.9-2.0, 2.1-2.3, 3.2-3.5 and 6.7-7.8; ^{13}C NMR ($CDCl_3$): 14.82, 39.46-40.28, 128-135.76, 150.68; ESI-MS: 751.1 (molecular ion peak) other peak 653, 518, 516, 366.1, 289.9 a.m.u; Anal. Calcd for [Cu ($C_{38}H_{38}N_{12}S_2Cl_2$)]$Cl_2$.2$H_2O$; C = 54.39; H = 6.28; N = 24.8; S = 14.2%. Found: C = 54.50; H = 6.32; N = 25; S = 14.6 %; μ_{eff} = 1.73-1.75 BM.

Cu (p-macehtsc)$_2$]Cl$_2$; bis (p-methoxyacetophenone thiosemicarbazone) copper II chloride (5)
Yield: 60 %; mp 205 °C; IR (KBr) (cm^{-1}): 3214, 1664, 1600, 1173; H^1 NMR (TMS) (ppm): 2.29, 2.51, 3.86, 6.8-7.0 and 7.7; ^{13}C NMR ($CDCl_3$): 26.27, 44.57, 113.63, 55.45, 129.60-131.45, 162.83; ESI-MS: 545.2 (Molecular ion peak) other peak 416, 399, 295, 250, 151.1 a.m.u.; Anal. Calcd for [Cu ($C_{40}H_{44}N_{12}O_2S_2$)]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 %; μ_{eff} = 1.73-1.76 BM.

[Cu (p-nacehtsc)$_2$]Cl$_2$; bis (p-nitroacetophenone thiosemicarbazone) copper II chloride (6)
Yield: 85 % mp 240 °C; IR (KBr) (cm^{-1}): 3342, 2990, 1691, 1605; H^{1}NMR (TMS) (ppm): 2.24, 2.3-2.5, 3.1, 7.0-8.2 and 8.9; ^{13}C NMR ($CDCl_3$): 20.42, 38.86-40.11, 43.81, 118.85-119.09, 123.22-129.15, 163.25-165.67; ESI-MS: 773.2 (Molecular ion peak) 771, 731.2, 508.1, 417.0, 377.1, 274.3, 248 a.m.u.; Anal. Calcd for [Cu ($C_{38}H_{38}N_{14}O_4S_2$)]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 %; μ_{eff} = 1.73-1.77 BM.

Biological evaluation

Cell Culture

The human breast carcinoma cell line MCF-7 was obtained from the National Center for Cell Science (NCCS), Pune, India. Cells were cultured in DMEM supplemented with 10% FBS, 100U/l Penicillin, 200mg/l Streptomycin & 50mg/l Gentamicin maintained at 37°C in a humidified 5% CO_2 Incubator.

For experiments, cells were trypsinized and cultured in 6-well (0.2 x 10^6 cells/well) and 96-well (1.0 x 10^4/well) plates initially for 48 h so as to allow the cells to attach. After 48 h, the cells were exposed to various concentrations (0.1-25µM) of the complexes (code 1-6) for the next 48 h. Each dose was tested in at least 3 replicate wells.

Cellular Morphological study

For the morphological analysis, cells in 6-well plate were observed under phase contrast microscope & photographed (Nikon Eclipse Ti, Japan).

Cell Viability and Cytotoxicity Assay

Sulforhodamine-B (SRB) assay was performed as reported earlier [49-50]. At the end of the incubation period, the cells were fixed with 10% TCA for 1 h at 4°C, and then washed three times with deionized water to remove TCA. Air-dried, TCA-fixed cells were stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. At the end of staining period, SRB was removed and washed with 1% acetic acid to remove unbound dye and air dried. The bound dye was dissolved with unbuffered 10 mM Tris base (pH=10.5). The absorbance was read at 560 nm in a SpectraMax Me2 Microplate Reader (Molecular Devices Inc.). Suitable untreated controls were also concomitantly employed.

Evaluation of Antibacterial Activity

The *in vitro* antibacterial effect of the complexes was evaluated against Gram-positive bacteria (*Staphylococcus aureus*) and four Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus vulgaris*) by the disc diffusion method [51] using nutrient agar medium. The bacteria were sub-cultured in the agar medium and were incubated for 24h at 37°C. The discs having a diameter of 5 mm, were then soaked in the test solutions (Sterile filter paper discs, Whatman No. 1.0) with the appropriate equivalent amount of the metal complexes dissolved in sterile dimethyl sulphoxide (DMSO) at concentrations of 1–10 mg/disc and were placed in petri dishes on an appropriate medium previously seeded with microbial organisms and stored in an incubator for the

above mentioned period of time. The inhibition zone around each disc was measured and the results recorded in the form of inhibition zones as a function of diameter (mm). To clarify any effect of DMSO on the biological screening, separate studies were carried out using DMSO as negative control where it showed no activity against any bacterial strains. Tetracycline was used as a positive control.

Evaluation of antioxidant activity (Hydrogen Peroxide Scavenging Capacity)
The ability of synthetic complexes to scavenge hydrogen peroxide (H_2O_2) was determined as reported previously [52]. A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH=7.4). Test solutions with the appropriate amount of the metal complexes (100µg/ml) dissolved in sterile DMSO were added to a H_2O_2 solution (0.6 mL, 40mM). Absorbance of H_2O_2 at 230 nm was determined 10 min later against a blank solution containing phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging by complexes was calculated as follows:

$$\% \text{ Scavenged } [H_2O_2] = [(A_C - A_S)/A_C] \times 100$$

Where A_C is the absorbance of the control and AS is the absorbance in the presence of the compounds [53].

RESULTS AND DISCUSSION

Biological Activity
All synthesized macrocyclic Cu (II) complexes were evaluated for their effectiveness against the human breast cancer cell line MCF-7 using Sulforhodamine B (SRB) cytotoxicity assay. For comparison purpose, the cytotoxicity of the standard antibreast cancer drug Tamoxifen was evaluated under the same experimental conditions. The values of cell viability were calculated after the tested compounds were incubated for 48 h. The IC_{50} values were calculated using SRB assay, as shown in Table 1. The order of cytotoxic activity was as follows: p-chloroacetophenone>p-nitroacetophenone> p-methoxy benzaldehyde > p-chloro benzaldehyde >p-nitro benzaldehyde>p-methoxy acetophenone thiosemicarbazide. The results obtained indicate that the activity of complexes increases by the presence of bulky groups bonded to N^4 of the thiosemicarbazone ligand. The complexes were found to have high activity of the order of 10 µmol/L thereby suggesting that the complexation of thiosemicarbazone to Cu (II) might be a good strategy to obtain antitumor agents. The similarity in the values of IC_{50} for the Cu (II) complexes is evidence in favor of the same biochemical action mechanism. In fact, there are several reports in literature that Cu (II) complexes of thiosemicarbazone derivatives are able to bind DNA *in* vitro [54] and present enhanced capacity to form inter-strand cross links as compared to cisplatin.

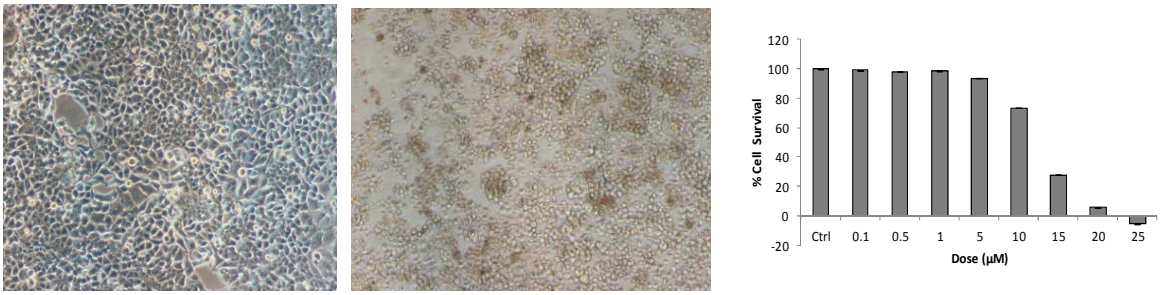
Cytotoxic Activity
Macrocyclic copper (II) chloride complexes 1-6 were screened for their potential anticancer/cytotoxic activity as shown in Table 1 using SRB assay. The standard antibreast cancer drug Tamoxifen was used as a positive control.

Table 1. Cytotoxic activity of compounds 1-6

Compound Code	Name of Compound	R	IC_{50} value
1.	$[Cu(C_{37}H_{36}N_{12}S_2Cl_2)]Cl_2$	R_1 =CH_3, R_2 = Cl and R_3=H	12µM
2.	$[Cu(C_{39}H_{42}N_{12}S_2O_2)]Cl_2$	R_1 =CH_3, R_2 = OCH_3 and R_3=H	8µM
3.	$[Cu(C_{37}H_{36}N_{14}S_2O_2)]Cl_2$	R_1 =CH_3, R_2 = NO_3 and R_3=H	12µM
4.	$[Cu(C_{38}H_{38}N_{12}S_2Cl_2)]Cl_2$	R_1 =CH_3, R_2 =Cl and R_3=CH_3	2µM
5.	$[Cu(C_{40}H_{44}N_{12}S_2O_2)]Cl_2$	R_1 =CH_3, R_2 = OCH_3 and R_3=CH_3	ND
6.	$[Cu(C_{38}H_{38}N_{14}S_2O_4)]Cl_2$	R_1 =CH_3, R_2 = NO_3 and R_3=CH_3	2µM

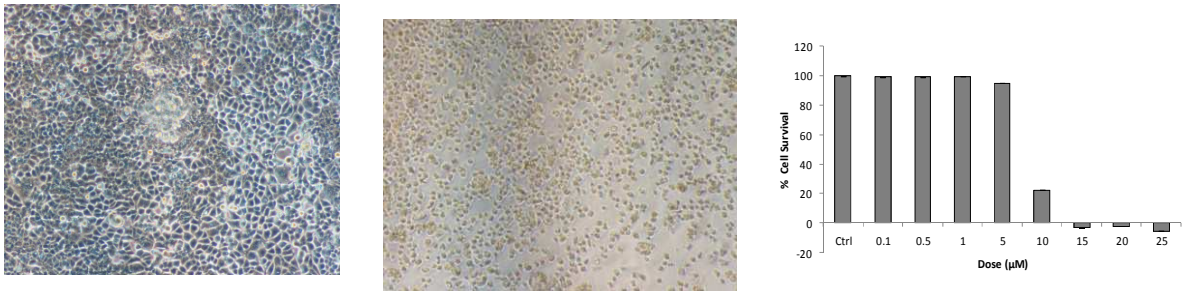
ND=Not Detected

Figs. 1-6 show the cytotoxicity assays for compounds 1-6. Compounds 1, 2, 3, 4 & 6 showed cytotoxic activity whereas complex 5 had no cytotoxic activity.



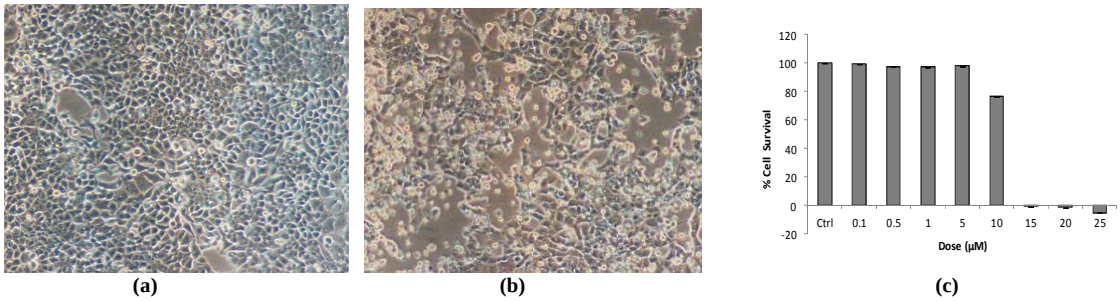
(a) **(b)** **(c)**

Fig.1 (a) Control showing untreated MCF-7 human breast cancer cells (b) Cytotoxic activity of Complex 1 at 12 µM. (C) Dose-dependent effect of Complex 1 on MCF-7 cells



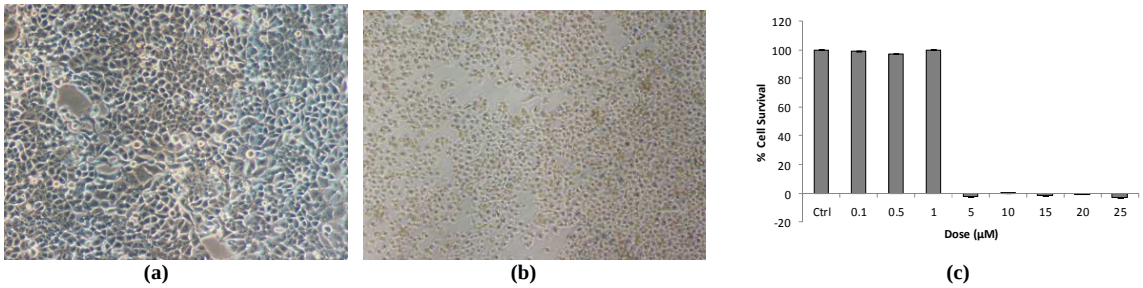
(a) **(b)** **(c)**

Fig.2 (a) Control showing untreated MCF-7 human breast cancer cells (b) Cytotoxic activity of Complex 2 at 8 µM. (C) Dose-dependent effect of Complex 2 on MCF-7 cells



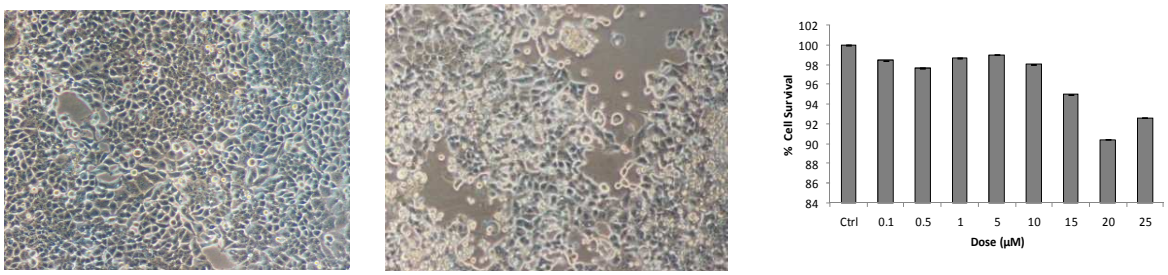
(a) **(b)** **(c)**

Fig.3 (a) Control showing untreated MCF-7 human breast cancer cells (b) Cytotoxic activity of Complex 3 at 12 µM. (C) Dose-dependent effect of Complex 3 on MCF-7 cells



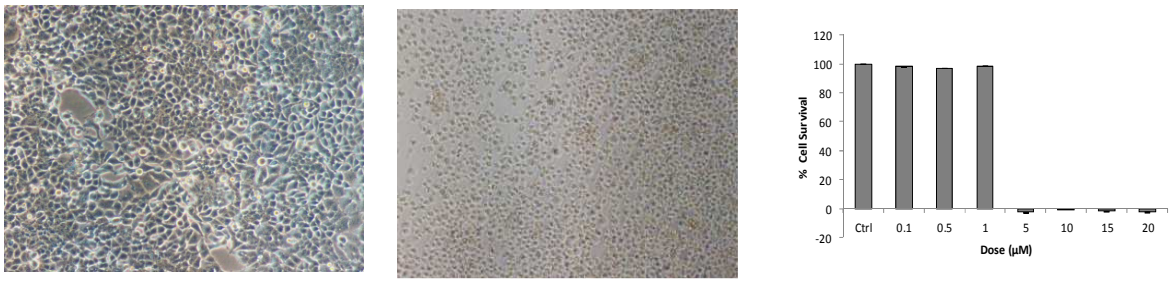
(a) **(b)** **(c)**

Fig.4 (a) Control showing untreated MCF-7 human breast cancer cells (b) Cytotoxic activity of Complex 4 at 2 µM. (C) Dose-dependent effect of Complex 4 on MCF-7 cells



(a) **(b)** **(c)**

Fig.5 (a) Control showing untreated MCF-7 human breast cancer cells (b) Cytotoxic activity of Complex 5 at 20 µM. (C) Dose-dependent effect of Complex on MCF-7 cells



(a) **(b)** **(c)**

Fig.6 (a) Control showing untreated MCF-7 human breast cancer cells (b) Cytotoxic activity of Complex 6 at 2 µM. (C) Dose-dependent effect of Complex 6 on MCF-7 cells

Antibacterial activity

The six macrocyclic copper (II) chloride complexes were also evaluated for their potential antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*. Tables 2-4 highlight the antibacterial activity of complexes 1-6 against *B. subtilis*, *S. aureus* and *E. coli* as observed by disc-diffusion method. None of the compounds were found to be active against *P. aeruginosa* at any concentration.

Table 2: Comparison of MIC values (in mg/ml) of Cu (II) complexes and standard antibiotic Tetracycline against *B. subtilis*

Compound Code	Ring Diameter (mm)						
	Tetracycline (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
4.	22	ND	8	10	20	12	9
5.	22	ND	8	16	12	8	8
6.	16	ND	ND	ND	ND	ND	22

ND= Not Detected

Table 3: Comparison of MIC values (in mg/ml) of Cu (II) complexes and standard antibiotic Tetracycline against *S. aureus*

Compound Code	Ring Diameter (mm)						
	Tetracycline (1mg/ml)	DMSO (1 mg/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
4.	18	ND	6	14	16	11	14
5.	12	ND	12	ND	ND	6	9
6.	18	ND	6	8	8	9	19

ND= Not Detected

Table 4: Comparison of MIC values (in mg/ml) of Cu (II) complexes and standard antibiotic Tetracycline against *E. coli*

Compound Code	Ring Diameter (mm)						
	Tetracycline (1mg/ml)	DMSO (1 mg/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
4.	16	ND	ND	11	8	8	12
5.	12	ND	ND	ND	ND	ND	6
6.	10	ND	ND	ND	ND	8	6

ND= Not Detected

The high antibacterial activity of copper (II) complexes may be due to co-ordination and chelation which tend to make metal complexes act as powerful and potent bacteriostatic agents, thus inhibiting the growth of the bacteria. In a complex, the positive charge on the metal is partially shared with the donor atoms present in the ligands and there may be delocalization of π electrons over the whole chelate. The increased activity of the metal chelates can be explained on the basis of chelation theory.

Antioxidant activity

Macrocyclic metal complexes have been suggested as promising agents for the diagnosis and treatment of different disease [55-57]. All compounds showed significant free radical scavenging action against peroxide induced release of free radicals at varying concentrations (200-1000 µg/ml). Ascorbic acid was used as a reference standard. The % scavenging as (mean ± SD) is shown in Table 5. In addition, some complexes have been suggested as a potential SOD mimics, mainly because of their high thermodynamic stability [58].

Table 5. *In-vitro* free radical scavenging effect of complexes 1-6 by peroxide scavenging method

S.No.	% Scavenging in µg/ml (Mean ± SD) of triplicates				
	Compound	200	400	800	1000
1	$[Cu(C_{37}H_{36}N_{12}S_2Cl_2)]Cl_2$	42.52 ± 0.028	45.55 ± 0.084	46.32 ± 0.151	48.72 ± 0.116
2	$[Cu(C_{39}H_{42}N_{12}S_2O_2)]Cl_2$	50.61 ± 0.056	53.85 ± 0.038	54.68 ± 0.036	58.68 ± 0.056
3	$[Cu(C_{37}H_{36}N_{14}S_2O_2)]Cl_2$	28.22 ± 0.083	31.24 ± 0.177	33.65 ± 0.200	40.62 ± 0.092
4	$[Cu(C_{38}H_{38}N_{12}S_2Cl_2)]Cl_2$	33.19 ± 0.036	36.96 ± 0.023	37.69 ± 0.092	42.68 ± 0.096
5	$[Cu(C_{40}H_{44}N_{12}S_2O_2)]Cl_2$	36.16 ± 0.046	35.96 ± 0.024	36.14 ± 0.044	35.94 ± 0.022
6	$[Cu(C_{38}H_{38}N_{14}S_2O_4)]Cl_2$	38.18 ± 0.082	37.18 ± 0.079	37.46 ± 0.093	38.10± 0.084

Spectroscopic Characterization

The main IR vibration bands of all macrocyclic Cu (II) complexes are shown in Fig.7. Upon co-ordination, change in the ν (C=S), ν(C=N) and ν (N-H) wave numbers, as compared to the values found for the thiosemicarbazone were observed for complexes 1-6. They were found to be consistent with the tridentate coordination of the thiosemicarbazone derivatives through the thiolate sulfur and azomethine nitrogen atoms [59]. The occurrence of the ν (N-N) band at higher frequencies in the IR spectra of the complexes as compared to those observed for the ligands, confirmed coordination through the azomethine nitrogen atom [60]. The ν (C=S) bands at 801-860 cm^{-1} in the spectra of free thiosemicarbazones shifted to the (782-786) cm^{-1} range in the complexes, thereby indicating coordination through the sulfur atom. These shifts to lower frequencies were found to be consistent with deprotonation and formation of a C-S single bond [61] (Fig. 7).

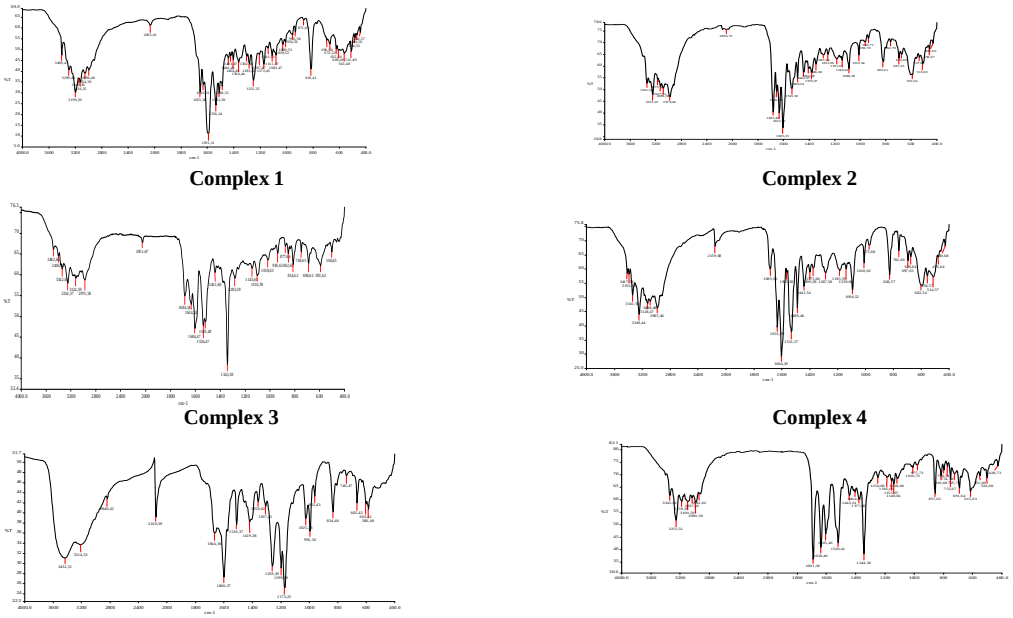


Fig. 7. IR Spectra of Complexes 1-6

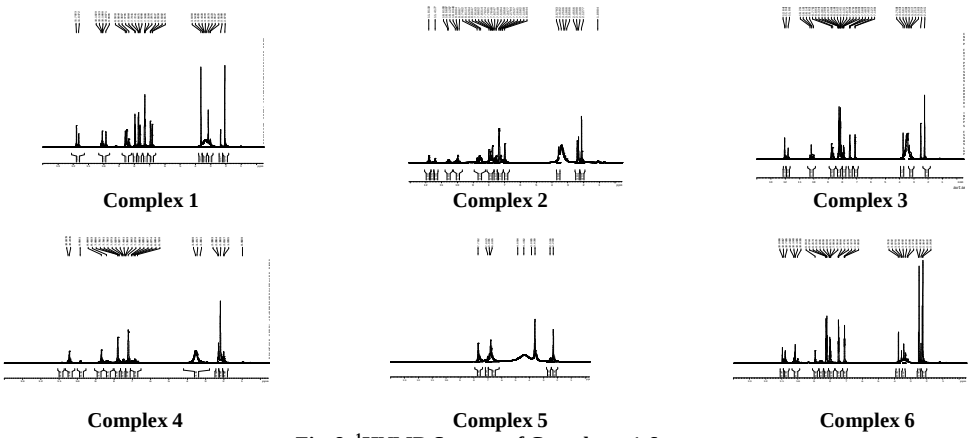


Fig. 8. ^{1}HNMR Spectra of Complexes 1-6

^{1}HNMR Spectra

The ^{1}HNMR spectra of the complexes were obtained in $CDCl_3$ at room temperature using TMS as an internal standard. The aromatic region showed 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 showed a sharp singlet at δ11.47 ppm. The multiplets observed in the region 6.81-7.93 ppm were assigned to the aromatic ring protons of carbohydrazone and the thiosemicarbazide moiety [62]. The H^{1}NMR spectra of metal complexes showed 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 metal chelates confirmed the participation of $–NH_2$ group and imino –NH group in the coordination with metal ions. 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 integration and signal multiplicity were found to be 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 ($C-CH_3$), as compared to the spectra of the thiosemicarbazones, confirmed coordination through the azomethine nitrogen atom. The data obtained from the electronic spectra of the complexes in CH_2Cl_2 solutions have been given in the experimental section.

Electronic spectra of Cu (II) complexes exhibited bands in the range 15,270-16,680 cm^{-1} and 18,200–19,200 cm^{-1} respectively corresponding to the transitions (Fig. 8).

^{13}CNMR spectra

The ^{13}CNMR spectra of synthesized macrocyclic Cu (II) complexes indicated new resonances at 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 corresponding to respective complexes 1-6 complexes. Fig. 9 depicts the respective ^{13}CNMR spectral assignments (ppm) of complexes 1-6.

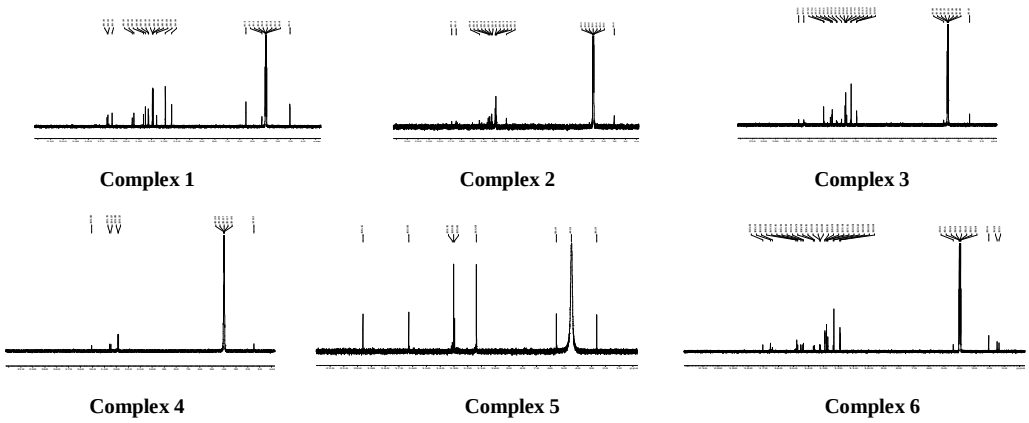


Fig. 9. ^{13}CNMR spectra of Complexes 1-6

CONCLUSION

This paper describes the synthesis and characterization as well as anticancer, antibacterial and antioxidant evaluation of the macrocyclic copper (II) complexes derived from the thiosemicarbazide. All Cu (II) macrocyclic complexes were synthesized and well characterized in detail by FTIR, ^{1}HNMR, ^{13}CNMR and LC-MS analysis. The Cu (II) complexes were in a distorted octahedral environment with the ligand having a tetradentate (C, N) chelating motif. Five of the six Cu (II) complexes showed significant *in-vitro* cytotoxic activity against human breast cancer cell line MCF-7. Further studies would entail studying the *in vitro* cytotoxic effect of the complexes against other cancer cell lines viz. lung, colon, ovarian etc., followed by *in vivo* studies in animal models as well as the *in vitro* effect of the complexes on various normal cell lines. More detailed studies are needed to understand the mechanisms of action at the cellular level and the role of the metal.

Cell shrinkage and rounding, membrane blebbing, chromatin condensation and nuclear fragmentation are important characteristics of apoptosis. In our study, prominent morphological changes, which are associated with apoptosis viz. live cell rounding, cell shrinkage and nuclear fragmentation, were observed when MCF-7 breast cancer cell line was treated with the macrocyclic Cu (II) complexes for 10 h. The data reported in this article might prove helpful guide for medicinal chemists working in this area.

Investigation of antibacterial screening data revealed that complexes 1-6 exhibited significant antibacterial activity against *B. subtilis*, *S. aureus* and *E. coli*.

All complexes were found to possess potent antioxidant activity in the range of 80-90% when screened for their radical scavenging activity against H_2O_2 [63-68]. Many present day diseases are reported to be due to an impaired balance of the pro-oxidant-antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either on account of increased generation of free radicals caused by excessive oxidative stress, or due to poor scavenging in the body caused by depletion of the dietary antioxidants. Reactive oxygen species differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission, modification of polypeptides, lipid peroxidation etc [52]. Antioxidants are the first line of defense against free radical damage, and are critical for maintaining optimum health. The need for antioxidants becomes even more critical with increased

exposure to free radicals. As part of a healthy lifestyle and a well-balanced, wholesome diet, antioxidant supplementation is now being recognized as an important means of improving free radical protection.

The macrocyclic ligands are highly significant in bioinorganic chemistry, catalysis as well as extraction of metal ions etc. Macrocyclic ligands in complex with transition metal ions show some interesting properties and biological functions such as being models for metalloproteinase and oxygen carrier systems. Keeping the above facts in mind and in continuation of our research work, the present paper reports the synthesis, characterization and evaluation of biological activity of macrocyclic Schiff base ligand complexes derived from the condensation of carbohydrazone with thiosemicarbazide and hydrated copper chloride. These complexes have the potential to emerge as leading candidates for drug development, if studied and screened further for their *in vivo* effects.

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REFERENCES

[1] K. Tanaka, A. Tengeiji, T. Kato, N.Toyama, M. Shiro, M. Shionoya**,** *J. Am. Chem. Soc.*, **2002**, 124, 12494.
[2] A.P. Kozikowski, W. Tuckmantel, G. Powis, *Angew Chem.*, **1992**, 31, 1379.
[3] L.J. Ming, *Med. Res. Rev.*, **2003**; 23, 697.
[4] D.L. Ma, C.M. Che, *Chemistry*, **2003**, 9, 6133.
[5]B. Lippert (Ed.), Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug, Verlag Helvetica Chimica Acta, Zürich, **1999**.
[6] A.S. AbuSurrah, M. Kettunen, *Curr. Med. Chem.*, **2006**, 13, 1337.
[7] C.S. Allardyce, P.J. Dyson, *Platinum Met. Rev.*, **2001**, 45, 62.
[8] I. Ott, R. Gust, *Arch. Pharm. Chem. Life*, **2007**, 340,117.
[9] M.A. Jakupec, M. Galanski, V.B. Arion, C.G. Hartinger, B.K. Keppler, *Dalton Trans.*, **2008**, 183.
[10] P.Yang, M. Guo, *Coord. Chem. Rev.*, **1999**, 185,189.
[11] S.K. Hadjikakou, N. Hadjiliadis, *Coord. Chem. Rev.*, **2009**; 253, 235.
[12] K. Strohfeldt, M.Tacke, *Chem. Soc. Rev.*, **2008**; 37:1174-1187.
[13] P.M. Abeysinghe, M.M. Harding, *Dalton Trans.*, **2007**, 3474.
[14] R. Gust, D. Posselt, K. Sommer, *J. Med. Chem.*, **2004**, 47, 5837.
[15] C.G. Hartinger, P.J. Dyson, *Chem. Soc. Rev.*, **2009**, 38, 391.
[16] M.B. Ferrari, F. Biscegliea, G.G. Favaa, G. Pelosia, P. Tarasconi, R. Albertini, S. Pinelli, *J. Inorg. Biochem.*, **2002**, 89, 36.
[17] M.B. Ferrari, F. Biscegliea, G. Pelosi, P. Tarasconia, R. Albertini, A. Bonati, P. Lunghi, S. Pinelli, *J. Inorg. Biochem.*, **2000**, 83,169.
[18] M.R. Arguelles, M.B. Ferrari, F. Biscegli, C. Pelizzi, G. Pelosi, S. Pinelli, M. Sassi, *J. Inorg. Biochem*., **2004**, 98, 313.
[19] M.B. Ferraria, F. Bisceglie, A. Buschini, S. Franzoni, G. Pelosi, S. Pinelli, P. Tarasconi, M. Tavone, *J. Inorg. Biochem.*, **2010**, 104, 199.
[20] D.X. West, E. Liberta, S.B. Padhye, R.C. Chikate, P.B. Sonawane, A.S. Kumbar, R.S. Yeranda, *Coord. Chem. Rev.*, **1993**, 123, 49.
[21] D.X. West, S.B. Padhye, P.B. Sonawane, *Struct. Bond.*, **1991**, 76, 150.
[22] Y. Haidue, A. Silverstru, *Coord. Chem. Rev.*, **1990**, 99, 253.
[23] O.E. Ichiro, D. Busch, H. Shull (Eds.), Bioinorganic Chemistry: An Introduction, Allym and Bacon, Boston, **1977**.
[24] R.W. Hay, J.R. Dilworth, K.B. Nolan, (Eds.), Perspectives on Bioinorganic Chemistry, JAI Press, London, **1991**.
[25] V.W.W. Yam, Y.L. Pui, W.P. Li, K.K.W. Lo, K.K. Cheung, *J. Chem. Soc., Dalton Trans.*, **1998**, 3615.
[26] M.R. Malachowski, B.T. Dorsey, M.J. Parker, M.E. Adams, R.S. Kelly, *Polyhedron*, **1998**, 17,1289.
[27] B.J. Hathaway, G. Wilkinson, R. Gillard, J.A. Mccleverty (Eds.), Comprehension Coordination Chemistry, Pergamon, Oxford, **1987**.
[28] D.W. Maragerum, G.D. Owens, H. Singel (Eds.), Metal ion in biological systems, Marcel Dekker, New York, **1981.**

[29] B. Dietrich, Design of Anion Receptors Applications, *Pure Appl. Chem.*, **1993**, 65, 1457.
[30] R.M. Izatt, K. Pawlak, J.S. Bardshaw, R.L. Bruening, *Chem. Rev.*, **1995**, 95, 2529.
[31] K. Kalcher, J.M. Kauffman, J. Wank, I. Vaneare, K. Vitras, C. Neuhal, Z. Yang, *Electro Analysis*, **1995**, 7, 5.
[32] M.A.T. Gilmartin, J.P. Hart, *Analyst.*, **1995**, 120, 1029.
[33] S. Chandra, K. Gupta, *Transit. Met. Chem.*, **2002**, 27,196.
[34] J. Liu, T.B. Lu, H. Deng, L.N. Ji, L.H. Qu, H. Zhou, *Transit. Met. Chem.*, **2003**, 28,116.
[35] T. Wang, Z.J. Guo, *Curr. Med. Chem.*, **2006**, 1315, 525.
[36] L.A. Saryan, E. Ankel, C. Krishnamurti, D.H. Petering, H. Elford, *J. Med. Chem.*, **1979**, 22, 1218.
[37] W.E. Antholine, J.M. Knight, D.H. Petering, *J. Med. Chem.*, 1976, 19, 339.
[38] B.A. Booth, K.C. Agrawal, E.C. Moore, A.C. Sartorelli, *Cancer Res.*, **1974**, 34, 1308.
[39] R.W. Brockman, J.R. Thomson, M.J. Bell, H.E. Skipper, *Cancer Res.*, **1956**, 16, 167.
[40] N. Raman, A. Sakthivel, K. Rajasekaran, *Mycobiol.*, **2007**, 35,150.
[41] S.A. Rice, M. Givskov, P. Steinberg, S. Kjelleberg, *J. Mol. Microbiol. Biotechnol.* **1999**, 1, 23.
[42] A. Ironmonger, B. Whittaker, J. Andrew, B. Baron, J. Chris, E. Alison, G. Ashcroft, A. Nelson, *Org. Biomol. Chem.*, **2007**, 5, 1081.
[43] G. Kumar, D. Kumar, S. Devi, R. Verma, R. Johari, *Int. J. Eng. Sci. Technol.*, **2011**, 3, 1630.
[44] K. Singh, M.S. Barwa, P. Tyagi, *Eur. J. Med. Chem.*, **2006**, 41,147.
[45] J.A. Obaleye, J.F. Adediji, M.A. Adebayo, *Molecules*, **2011**, 16, 5861.
[46] X. Tai, X. Yin, Q. Chen, M. Tan, *Molecules*, **2003**, 8, 439.
[47] R. Kothari, B. Sharma, *Int. J. Chem.*, **2013**, 2,199.
[48] A.A.H. Kadhum, A.B. Mohamad, A.A. Al-Amiery, M.S. Takriff, *Molecules*, **2011**, 16, 6969.
[49] M. Nigam, V. Ranjan, S. Srivastava, R. Sharma, A.K. Balapure, *Life Sci.*, **2008**, 82, 577.
[50] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T.Warren, H. Bokesch, S. Kenney, M.R. Boyd, *J. Natl. Cancer Inst.*, **1990**, 82, 1107.
[51] A.A. Al-Amiery, A. Mohammed, H. Ibrahim, A. Abbas, *J. Biotechnol. Res. Cent.*, **2010**, 4, 55.
[52] R.J. Ruch, S.J. Cheng, J.E. Klaunig, *Carcinogenesis*, **1989**, 10, 1003.
[53] A.K.Tiwari, *Curr. Sci.*, **2001**, 81, 1179.
[54] A.G. Quiroga, J.M. Perez, E.I. Montero, J.R. Masaguer, C. Alonso, C.N Ranninger, *J. Inorg. Biochem.*, **1998**, 70,117.
[55] X. Liang, P.J. Sadler, *Chem. Soc. Rev.*, **2004**, 33, 246.
[56] F. Marques, L. Gano, M.P. Campello, S. Laceida, I. Santos, L.M. Lima, J. Coster, P. Antunes, R. Delgado, *J. Inorg. Biochem.*, **2006**, 100, 270.
[57] F. Marques, K.P. Guerra, L. Gano, M.P. Campello, I. Santos, L.M. Lima, J. Coster, P. Antunes, R. Delgado, *J. Biol. Inorg. Chem.*, **2004**, 9, 859.
[58] D.P. Riley, *Chem. Rev.*, **1999**, 99, 2573.
[59] D.L. Klayman, J.F. Bartosevich, T.S. Griffin, C.J. Manson, J.P. Scovill, *J. Med. Chem.*, **1979**, 22, 855.
[60] A.P. Rebolledo, M. Vieites, D. Gambino, O.E. Piro, E.E. Castellauo, C.L. Zani, E.M. Souza Fagundes, L.R. Teixeira, A.A. Batesta, A.A. Beraldo, *J. Inorg. Biochem.*, **2005**, 99, 698.
[61] D.D. Kovala, A. Domopoulou, M.A. Demervzis, G. Valle, A. Papageorgiou, *J. Inorg. Biochem.* **1997**, 68,147.
[62] B. Halliwell, J.M. Gutteridge, *Arch. Biochem. Biophys.*, **1986**, 246, 501.
[63] A. Corona-Bustamante, J.M. Viveros-Paredes, A. Flores-Parra, A.L. Peraza-Campos, F.J. Martínez-Martínez, M.T. Sumaya-Martínez, A. Ramos-Organillo, Molecules, **2010**, 15, 5445.
[64] N.H. Al-Sha'alan, Molecules, **2007**, 12, 1080.
[65] S. Chandra, D. Jain, A.K. Sharma, P. Sharma, *Molecules*, **2009**, 14, 174.
[66] K.S. Prasad, L.S. Kumar, M. Prasad, H.D. Revanasiddappa, *Bioinorg. Chem. App.*, **2010**, Article ID 854514.
[67] H.L. Singh, A.K.Varshney, *Bioinorg. Chem. App.*, **2006**, Article ID 23245.
[68] M. Alkan, H. Yuksek, O. Gursoy-Kol, M. Calapoglu, Molecules, **2008**, 13,107.