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Original Article

Synthesis, spectroscopic characterization and pharmacological evaluation of novel copper mediated antibiotics

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ABSTRACT

A series of novel copper mediated antibiotics were prepared from N^4 – substituted having structure [Cu $(L)_2$] Cl_2. All compounds were characterized by various physico -chemical techniques like melting point, TLC, elemental analysis, IR, H^1-NMR, LC-MS, UV-Visible spectroscopy. The magnetic moments and electronic spectral studies suggested distorted octahedral geometry for all the compounds. The monoanionic orthophenylene diammine ligands were in a bidentated mode, binding through azomethine nitrogen atoms of substituted bis carbohydrazones. The synthesized compounds were screened for their in vitro antibacterial activity using disc diffusion method against two strains of gram negative and gram positive bacteria. Streptomycin antibiotic was used as positive control. All compounds showed significant antibacterial activity in the range of 1-10 mg/ml. Invitro antioxidant activity of the all copper compounds was screened by using the DPPH scavenging assay by using ascorbic acid as a positive control. All compounds exhibited potent antioxidant activity in the range 80-90%. . The Invitro-cytotoxic activity of compounds against breast cancer cell lines MCF-7 was evaluated for all the above compounds. All compounds were found to be highly active against the studied cell line; presenting the similar values of IC_{50} around 10 mmol/L. These compounds would be evaluated further for their possible DNA binding, cleavage, antifungal and anti-diabetic properties.

Keywords - Copper mediated antibiotics, Spectroscopic characterization**,** Pharmacological evaluation, Invitro-antibacterial, antioxidant activity and anti -cancer activity.

INTRODUCTION

Cancer is undoubtedly one of the main health concerns facing our society and one of the primary target of medicinal chemistry. It is the second leading cause of death in economically developed countries and the third cause of death in developing countries. Discovery for new types of anticancer drugs is in process and the mechanism of interaction of such drugs with DNA is under exploration. Cisplatin (cis-diammine dichloroplatinum (II)) is a widely used chemotherapeutic agent for the treatment of testicular cancer and it is used in combination regimens for a variety of other tumors, including ovarian, cervical, bladder, lung and those of the head and neck. Despite the success of cisplatin, problems regarding intrinsic or acquired resistance and side effects have encouraged the development of new platinum drugs.[1] Even though platinum-based complexes had been in primary focus of research on chemotherapy agents, the interests in this field have shifted to non-platinum-based agents, in order to find different metal complexes with less side effects and similar, or better Cytotoxicity . The choice of metal ion is the most important factor in the design of metal-based chemotherapeutic agent. Varieties of metal complexes have been used as drugs and are well known to increase their activity [2].

Cu(II) is an essential and bio-relevant element[3] Cu(II) for normal metabolism because of its functions as cofactor of several metallo -enzymes. Copper is widely distributed in the biological system and copper complexes are known to have a broad spectrum of biological action. It has been demonstrated that Cu accumulates in tumors due to selective permeability of the cancer cell membranes. Because of this, a number of copper complexes have been screened for anticancer activity and some of them were found active both *in vivo* and *in vitro*.[4] Furthermore, copper(II)-based complexes appear to be very promising candidates for anticancer therapy; an idea supported by a considerable number of research

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articles describing the synthesis and cytotoxic activities of numerous copper(II) complexes [5]. Cis – Diamminedichloro platinum (II) cisplatin [6] is one of the most widely used and effective oncological agents against cancers of the testicles, ovaries, bladder, head and neck [7-9]. It is also an important drug for cancers of the lung, cervix and breast. It's most impressive success has been in the treatment of testicular cancer. However, its clinical usefulness has frequently been limited by various side effects [10-11], such as nephrotoxicity, ototoxicity and neurotoxicity and by the emergence of cancer cells resistant to cisplatin. Cis – Diammine (1, 1 – cyclobutane dicarboxylato platinum (II) (carboplatin) [12-14] is the only clinically successful second – generation platinum complex, being less nephrotoxic and emetogenic than cisplatin. These properties have been attributed to the greater pharmacokinetic stability of its 1, 1 – cyclobutane dicarboxylate ligand in solution [15-16]. In recent years, extensive efforts have been made to develop third generation platinum complexes with a broader spectrum of activity; to improve clinical effectiveness, lack of cross – resistance to cisplatin and enhance water solubility [17]. Since some substituted orthophenylenediammine platinum complexes have shown antitumor activity against a variety of cell tumor [18-19] and some aromatic compounds have shown the possibility of intercalation between bases [20]. The numerous Schiff bases and their metal complexes have been investigated and structure of that complexes also discussed; which is synthesized from orthophylenediammine in which orthophylenediammine work as classic ligand [21-22]. Orthophylenediammine is primary amine that can react with aldehyde ketone and form the Schiff bases ligand which contain (>C=N-) type group that can react with numerous metal salt and form the coordination complexes. The coordination complexes of copper (II) have been found stable complexes; in which orthophylenediammine act as ligands and studied by various method [23-24]. Literature [25-26] reveals its steric hinderence in the formation of coordination complexes. Copper plays an important role in those reactions which carried out catalytically in human being or any other living organism and it has strong capability to formed the coordinated complexes with amino acids, peptides, proteins and other enzymes [27-28]. Copper also take part in various electron – transfer reaction and oxygen transport process in protein; azurin, plastocynin and laccase. Amino acids take part in the foundation of living organism; other chemical compounds do not play an important role in the formation of living organism as compared to amino acids. The copper complexes showed protonation and stability constants that are similar to bio-ligands in biological system [29]. On the basis of above explanation; it has been explained that in-vivo media can be demonstrated by aqueous solution. However, literature reveals that this media is not suitable for in-vivo reaction in aqueous solution and media is necessary in non-aqueous solution that contains lipophilic character [30-31]. The coordinated copper (II) complexes of amino acids attracted a lot because copper ions showed transport property in living things [32]. Numerous binary and ternary amino acid copper (II) ion and other divalent transition metal ions have been synthesized in solution as well as in the solid state [33-36].

In this paper, we have discussed the in vitro anticancer activity of the Copper(II) complexes specifically against the human breast cancer MCF-7 cell line, that can be useful for developing better anticancer and anti-tumor drug with copper (II) complexes with special emphasis on the identification of structure-activity relationships for the different classes of copper (I, II) complexes.

MATERIALS AND METHODS

Chemistry: All glass wares 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 gel60F254 plates with iodine vapors as detecting agent. Tetra methyl silane (TMS; 0.0 ppm) was used as an internal standard in 1HNMR. 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. 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 bis carbohydrazones of complexation agent were prepared by standard methods described in the literature [30].

Synthesis of Novel Copper mediated antibiotics: A mixture of hydrated copper chloride, substituted biscarbohydrazons (1-5) and orthophenylene diammine hydrochloride in 2:2:1 molar ratio in absolute ethanol were added slowly with stirring in a round bottom flask at 70-80^{0}c for 8 hours.

Solvent was evaporated under reduced pressure and the residue obtained was quenched with ethanol. The solid product was precipitated, filtered off, washed several times with cold ethanol and dried over fused $CaCl_2$ in desiccators. A good yield of product was obtained and the purity of the complex was confirmed by the TLC and the elemental analysis.

A.[Cu(p-macehorthophen)$_2$]Cl$_2$;bis(p-methoxyacetophenone orthophenylenediammine) copper II chloride: Yield: 70 %; mp 275°C; IR (KBr) (cm-1): 3296, 3038, 1580, 536; ^{1}H-NMR (TMS) (ppm): 2.58, 2.67-2.68, 3.96-4.12 and 6.8-6.968; ESI MS: 909.5 (observed peak) 791.2, 730, 222.1, 154.1 a.m.u.; Anal. Calcd for [Cu($C_{48}H_{50}N_{10}O_2$)]Cl_2 ; C = 58.90; H = 5.80; N = 16.62. Found: C = 57.20; H = 5.97; N = 16.76; λ_{max} = 378; Molar conductance = 42.6 ohm^{-1}cm^{2}mho^{-1}

B.[Cu(p-nacehorthophen)$_2$]Cl$_2$;bis(p-nitroacetophenone orthophenylenediammine)) copper II chloride: Yield: 66%; mp 248 °C; IR (KBr) (cm-1): 3233, 3039, 1585, 538; ^{1}H-NMR (TMS) (ppm): 1.4, 2.1-2.28, 3.65-3.67, 7.38-8.14; ESI-MS: 939.5 (molecular ion peak) 824.3, 512.4, 400.8, 310.3, 104.23 a.m.u; Anal. Calcd for [Cu($C_{46}H_{44}N_{12}O_4$)]Cl_2; C = 53.88; H = 4.96 N = 18.90. Found: C = 53.86; H = 4.74; N = 17.91; λ_{max} = 392; Molar conductance = 38.45ohm^{-1}cm^{2}mho^{-1}

C.[Cu(p-clbhorthophen)$_2$]Cl$_2$; bis (p-chlorobenzylidene orthophenylenediammine)) copper II chloride: Yield: 70 %; mp 3250 °C; IR (KBr) (cm-1): 3298, 3039, 1624, 496; ^{1}H-NMR (TMS) (ppm): 2.2, 2.25-2.346, 3.14-3.16 and 6.80-6.86 ; ESI-MS: 894..3 (molecular ion peak) 797.1, 492.2, 283.2, 105.0 a.m.u; Anal. Calcd for [Cu($C_{44}H_{40}N_{10}Cl_2$)]Cl_2; C = 54.16; H = 4.78; N = 16.64. Found: C = 54.1; H = 4.36; N = 16.24. λ_{max} = 388; Molar conductance = 53.45ohm^{-1}cm^{2}mho^{-1}

D.[Cu(pmbhorthophen)$_2$]Cl$_2$;bis(p-methoxybenzylidene orthophenylenediammine)) copper II chloride: Yield: 70 %; mp 265 °C; IR (KBr) (cm-1): 3288, 2995, 1610, 526; ^{1}H-NMR (TMS) (ppm): 1.6, 2.44-2.50, 3.44 and 7.22-7.38; ESI-MS: 879.1 (Molecular ion peak) 666.1, 409.1, 198.2 a.m.u.; Anal. Calcd for [Cu ($C_{46}H_{46}N_{10}O_2$)] Cl_2; C = 57.68; H = 5.59; N = 16.88. Found: C = 57.42; H = 5.29; N = 16.86; λ_{max} = 398; Molar conductance = 25.16 ohm^{-1}cm^{2}mho^{-1}

E.[Cu(p-nbhorthophen)$_2$]Cl$_2$;bis(p-nitrobenzylidene orthophenylenediammine)) copper II chloride: Yield: 80 % mp 296 °C; IR (KBr) (cm-1): 3369, 3130, 1598, 540; ^{1}H-NMR (TMS) (ppm): 2.18, 2.55, 3.18-3.62 and 7.49 – 8.18; ESI-MS: 780.1 (Molecular ion peak) 561.1, 374.2, 220.1 a.m.u.; Anal. Calcd for [Cu ($C_{44}H_{40}N_{12}O_4$)] Cl_2; C = 52.88; H = 4.65; N = 19.60. Found: C = 52.18; H = 4.61; N = 19.50; λ_{max} = 440, Molar conductance = 28.35ohm^{-1}cm^{2}mho^{-1}

Biological Activity

In-vitro Antibacterial Activity: The *in vitro* antibacterial effects of copper complexes were evaluated against two sp. of Gram-positive bacteria (*Staphylococcus aureus (MTCC 3160) and B. Subtilis (MTCC 1134))* and two Gram-negative bacteria (*Escherichia coli (MTCC 50), Pseudomonas aeruginosa (MTCC 1034*) by the disc diffusion method using nutrient agar medium. The bacteria were sub-cultured in the agar medium and were incubated for 24 h at 37 °C. The discs having a diameter of 5 mm, were then soaked in the test solutions (Sterile filter paper discs, What man No. 1.0) with the equivalent amount of compounds dissolved in sterile dimethylsulphoxide (DMSO) at concentrations of 10 mg/ml and were placed in petri dishes on an appropriate medium previously seeded with microbial organisms and stored in an incubator for 24 hrs. The inhibition zone around each disc was measured and the results were recorded in the form of inhibition zones (diameter, mm). To clarify any effect of DMSO on the biological screening, separate studies were carried out using DMSO as negative control and it showed no activity against any bacterial strains. Streptomycin antibiotic was used as a positive control in this antibacterial analysis.

In-vitro Antioxidant Activity: The free radical scavenging activity (RSA) of copper complexes at concentration 200, 400,600, 800, 1000 µg/ml was carried out in presence of freshly prepared solution of stable free radical DPPH (0.04% w/v) following Hataro's method 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-

$$\%RSA = \frac{A_C - A_S}{A_C} \times 100$$

Where, A_C = Absorbance of control. A_S = Absorbance of test Sample

In-vitro Anticancer Activity: The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using DMEM

containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37° C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50 µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line [119].

The Human Breast Carcinoma Cell Line; MCF-7 Cells were 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 (0.2×10^6 cells/well) and 96-well (1.0×10^4/well) plates initially for 48 h so as to allow the cells to attach. After 48 h, the cells were exposed to various concentrations of complexes for the next 48 h. Each dose was tested in at least triplicate wells

RESULT AND DISCUSSION

All copper mediated antibiotics were synthesized by the template method. Substituted bis-carbohydrazone, orthophenylenediammine and $CuCl_2.2H_2O$ were taken in 2:2:1 molar ratio in round bottom flask. All the complexes are stable to the atmosphere and had high melting points. Elemental analysis; C, H and N of the complexes were evaluated from SAIF, Punjab University, Chandigarh and the low molar conductance values of all the complexes in DMSO at room temperature indicated them to be non-electrolytic in nature. All complexes are completely soluble in DMF, DMSO and ethanol but insoluble in water. The copper content was determined by the EDTA titration method

Table 1 Physical and Analytical data of novel copper mediated antibiotics

Compounds	Molecular Formula	Color	Yield	M.P. (°C)	Mol.wt.	Molar conductance $\Omega^{-1}cm^2mol^{-1}$	Analysis (%) Found (Calcd.)			λ_{max}
							C%	H%	N%	
A	$[Cu(C_{42}H_{50}N_{10}O_2)]Cl_2$	Green	65	225	860.54	46.5	57.84/58.56	5.97/5.81	15.76/16.26	365 nm
B	$[Cu(C_{40}H_{44}N_{12}O_4)]Cl_2$	Green	76	240	890.54	38.2	53.56/53.89	4.73/4.94	17.51/18.86	390 nm
C	$[Cu(C_{38}H_{40}N_{10}Cl_2)]Cl_2$	Green	40	310	841.54	53.3	54.21/54.18	4.26/4.75	16.79/16.63	386 nm
D	$[Cu(C_{40}H_{46}N_{10}O_2)]Cl_2$	Cream	60	165	832.54	23.1	57.12/57.64	5.89/5.52	16.82/16.81	395 nm
E	$[Cu(C_{38}H_{40}N_{12}O_4)]Cl_2$	Greenish yellow	48	230	862.54	26.2	51.78/52.86	4.77/4.63	19.37/19.47	410 nm

Table 2 The Relevant IR peaks of Novel Copper mediated antibiotics

S. No.	Compounds	ν(N-H)	ν(Ar-H)	ν (C=N)	ν (Cu-N)
1.	A	3296	3039	1582	534
2..	B	3234	3033	1584	538
3.	C	3294	3036	1626	498
4.	D	3288	2968	1610	528
5.	E	3242	3036	1598	534

Spectroscopic Characterization

Infrared Spectra: The main IR vibration bands of all copper compounds are listed in Table 2. Upon coordination, change in the frequency ν (C=N) and ν (N-H) wave numbers in comparison to the values found for the antibiotics a-e. They are consistent with the tetra dentate coordination of the substituted bis carbohydrazones ligand through the azomethine nitrogen atoms.Upon co-ordination, change in the ν(C=N) and ν (N-H) wave numbers, as compared to the values found for the primary amine were observed for complexes a-e. They were found to be consistent with the bidentate coordination of the orthophenylenediammine derivatives through the azomethine nitrogen atoms [37]. The occurrence of theν (N-N) band at higher frequencies in the IR spectra of the compounds as compared to those observed for the ligands, confirmed coordination through azomethine nitrogen atoms [38].

1HNMR Spectra

The 1HNMR spectra of the compounds 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 6.85-7.96 ppm were assigned to the aromatic ring protons of bis carbohydrazone and the orthophenylenediammine moiety [40]. The H1NMR spectra of copper compounds showed signals corresponding to –CH3, –NH2, –NH (hydrazone) and –OH protons at 2.26 (5, 3H), 7.41-7.44 (M, 3H), 8.059-8.40 (2H), 10.06 (s, 1H) and 11.86 (s, 1H), respectively. The NMR spectrum of copper compounds confirmed the participation of –NH2 group and imino –NH group in the coordination with copper ions. Some hydrogen atom values of δ were not observed precisely due to overlapping with the signals of the aromatic hydrogen atoms of substituted biscarbohydrazone ligand. 1HNMR integration and signal multiplicity were found to be in agreement with the proposed structures. In the 1HNMR spectra of the compounds a high frequency shift of Ca (0.13 ppm), for the methyl hydrogen atoms (C-CH_3), as compared to the spectra of the ligands, confirmed coordination through the azomethine nitrogen atom. Electronic spectra of Cu (II) complexes exhibited bands in the range 15,279-16,690 cm-1 and 18,220–19,240 cm^{-1} respectively corresponding to the transitions .The main peaks observed in spectra are given in table 3.

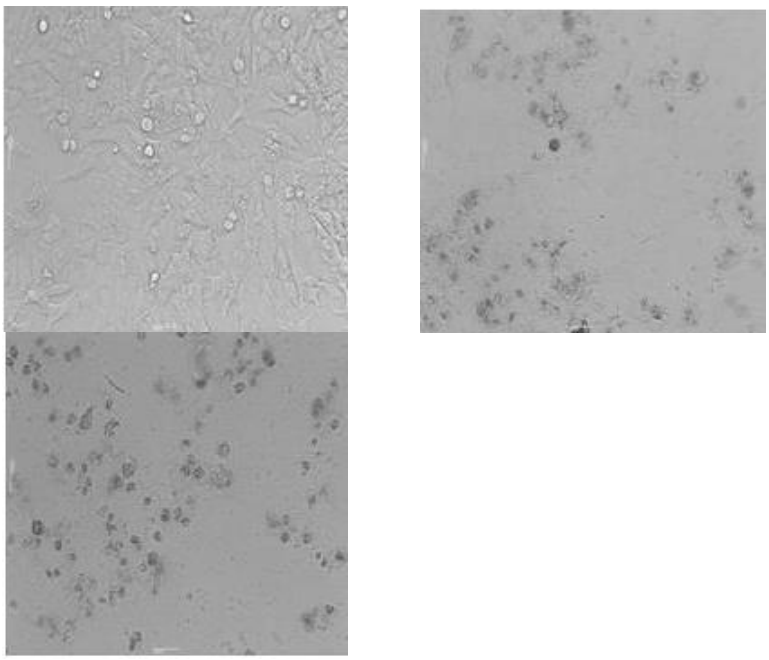
Table 3 The Relevant ^{1}H-NMR peaks of Novel Copper mediated antibiotics

S.No.	Compounds	δ - CH_3	δ -CH_2	δ -NH	δ - C_6H_5
1.	A	2.59	2.69-2.70	3.94-4.06	6.74-6.96
2.	B	1.4	2.2-2.26	3.62-3.68	7.45-8.06
3.	C	2.6	2.22-2.36	3.14-3.16	6.86-7.10
4.	D	1.9	2.50-2.54	3.44- 3.46	7.14 – 7.39
5	E	2.17	2.54	3.18-3.20	7.46-8.19

In-vitro Anticancer Activity: The all compounds were evaluated for their effectiveness against the human breast cancer cell line MCF-7 using MTT cytotoxicity assay. For comparison purpose, the cytotoxicity of the standard anti-breast cancer drug Tamoxifen was used under the same experimental conditions. The IC_{50} value was calculated using MTT assay, as shown in Table 4. The results revealed that the activity of compounds increases by the presence of bulky groups bonded to N4 of the orthophylenediammine moiety. The compounds were found to have high activity. 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, the literature reports that Cu (II) complexes of orthophylene diammine derivatives are able to bind DNA *in vitro* and present enhanced capacity to form inter-strand cross links as compared to cisplatin. Result revealed that compounds were exhibit potent activity against the MCF – cell line.

Table 4: Cytotoxic activity of Novel Copper mediated antibiotics against MCF-7 breast cancer cell line

S.No.	Compounds	Test Conc. (%)	% Cytotoxicity	CTC_{50} (µg/ml)
1.	A	1000 500 250 125 62.5	78.62±0.2 78.43±1.0 76.04±0.6 44.08 ±4.2 19.52±5.9	145.22±8.9
2.	B	1000 500 250 125 62.5	78.68±0.3 78.50±1.0 76.06±0.6 44.09±4.4 19.58 ± 6.0	146.24±8.9
3.	C	1000 500 250 125 62.5	78.65±0.4 78.52±1.5 76.06±0.8 44.09±4.1 19.52±5.3	144.00±8.7
4.	D	1000 500 250 125 62.5	78.76±0.34 78.58±1.4 76.24±0.5 44.05±4.6 19.56±5.9	145.00±8.7
5.	F	1000 500 250 125 62.5	78.64±0.6 78.50±1.5 76.09±0.7 44.09±4.6 19.53±5.4	145.20±8.8



Control – MCF 7 Compound a 1000 µg/ml Compound a 500 µg/ml

Figure1 : Antineoplastic activity of compound (a) against MCF – 7 Breast cancer cell line

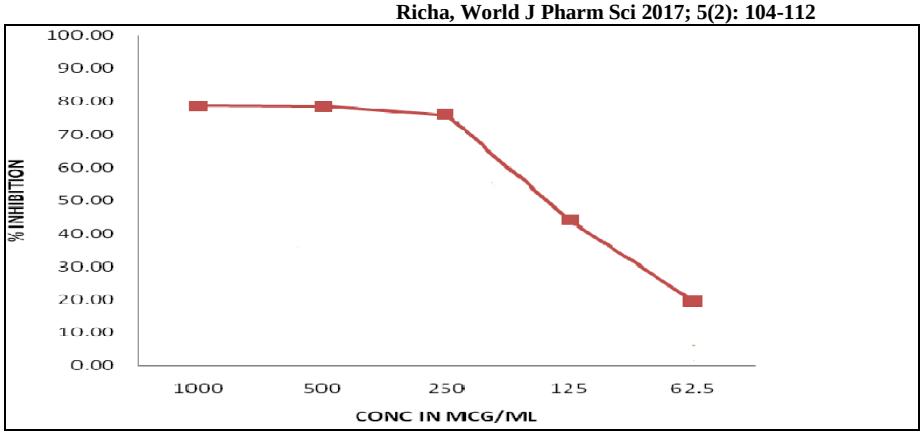


Figure 2: Graph shows anticancer activity against MCF – 7 Breast cancer cell line

In-vitro Antibacterial Activity: The all copper (II) complexes were also evaluated for their potential antibacterial activity against *B. subtilis (MTCC 1134), S. aureus (MTCC 3160), E. coli (MTCC 50)* and *P. aeruginosa (MTCC 1034)*. Tables 5 highlight the antibacterial activity of complexes a-e against *B. subtilis, S. aureus, E. coli and P. aeruginosa* as observed by disc-diffusion method. The high antibacterial activity of copper (II) compounds may be due to coordination and chelation which tend to make copper compounds act as powerful and potent bacteriostatic agents, thus inhibiting the growth of the bacteria. In a compound, the positive charge on the copper 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 compounds can be explained on the basis of chelation theory. The result of the this series revealed that the all copper (II) compounds contain significant antibacterial activity against two gram positive bacteria and two gram positive bacteria. All the copper compounds exhibit higher antibacterial activity against the P. aurogenosa bacteria. All the copper (II) compounds showed the less antibacterial activity in compared to standard drug streptomycin.

Table 5 Invitro antibacterial activities of all copper compounds against gram positive and gram negative bacteria

Compounds	Zone of inhibition (in mm) and Concentration 1mg/ mL of various strains			
	E. coli	*P. aerogenosa*	*B. subtilis*	*S. aureus*
A	13	22	15	12
B	14	25	15	13
C	9	18	6	8
D	10	16	5	6
E	8	10	10	9
Streptomycin (Standard antibiotic drug)	18	28	20	22

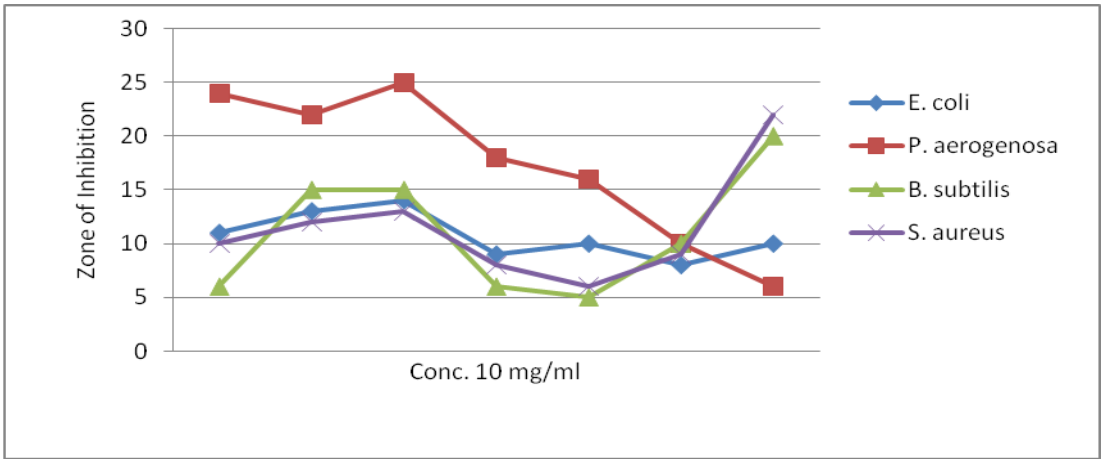


Figure 3. Graph represents theInvitro- antibacterial activity of Cu (II) compounds

Mode of Action: The chelation theory accounts for the increased activity of the metal compounds. The chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and posible π-electron delocalisation within the whole chelating ring. The chelation increases the lipophilic nature of the central atom which subsequently favours its permeation through the lipid layer of the cell membrane. The degradative enzymes produced by the microorganism are important in host infection. For food deterioration and break down of organic matter. The enzyme production is here intended to mean both synthesis of the enzyme by the microorganisms and activity of the enzyme in the medium after it is produced. Since the metal complexes inhibit the growth of microorganism it is assumed that the production of enzyme is being affected and hence the microorganism is unable to utilize the food for itself or the intake of nutrients in suitable forms decreases and consequently the growth of microorganism is arrested, while higher concentration proves fatal. The higher concentration destroys the enzyme mechanism by blocking any of the metabolism path way and due to the lack of availability of proper food, the organism dies. The results of biological activity have been compared with the conventional antibiotic streptomycin used as standard.

In-vitro Antioxidant Activity: The copper (II) compounds have been suggested as promising agents for the diagnosis and treatment of different disease. 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 is shown in Table 6. In addition, some complexes have been suggested as a potential SOD mimics, mainly because of their high thermodynamic stability. Table 6 revealed that the all compounds showed the significant antioxidant activity at the concentration of 1000 µg / ml.

Table 6 *In-vitro* free radical scavenging effect of novel copper mediated antibiotics

S.No.	Compounds	% Scavenging of triplicates				
		200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml
1.	A	25	39.5	45.3	46	74
2.	B	22.5	37.9	52.2	44.3	73.3
3.	C	14.6	34.13	57.3	42.5	72.0
4	D	21.8	35.54	62.1	42.5	72.63
5.	E	20.17	35.67	60.1	42.65	72.63
6.	Ascorbic acid(Standard)	50	65.4	68.2	75.3	80.6

The antioxidant activity of all the copper (II) compounds given in table 6, the order of antioxidant activities of all complexes are as: b > c > e> d > a. All the copper (II) compounds showed the significant antioxidant activity against standard ascorbic acid. All the copper (II) compounds exhibit significant antioxidant activity but less than control ascorbic acid.

CONCLUSION

The copper compounds were in a distorted octahedral environment with the ligand having a tetradentate (C, N) chelating motif. All compounds showed significant *in-vitro* cytotoxic activity against human breast cancer cell line MCF-7. The *in vitro* cytotoxic effect of the copper complexes against cancer cell lines viz. lung, colon, ovarian etc., followed by *in vivo* studies in animal models were evaluated. More detailed studies are needed to understand the mechanisms of action at the cellular level and the role of the metal. 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 copper compounds for 10 h. The data reported in this paper may be used by medicinal chemists working in this area. Investigation of antibacterial screening data revealed that compounds exhibited significant antibacterial activity against *B. subtilis*, *S. aureus* and *E. coli*. *P. aerogenosa*. All compounds were found to possess potent antioxidant activity in the range of 80-90% when screened for their radical scavenging activity against DPPH assay.

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REFERENCES

1. Gocke M, Utku S, Gur S, Rozkul A, Gumus F. Synthesis, in vitro cytotoxic and antiviral activity of cis -[Pt(R(–) and S(+)-2-a-hydroxybenzylbenzimidazole)2Cl2] complexes. Eur. J. Med. Chem, 2005; 40: 135.
2. Moriuchi T, Nomoto A, Yoshida K, Hirao T. Characterization of ferrocene derivatives bearing podand dipeptide chains (-l-Ala-l-Pro-OR). J. Organomet. Chem, 1999; 589: 50-58.
3. Fei BL, Xu WS, Tao HW, Li,W, Zhang Y, Long JY, Liu QB, Xia B, Sun WY. Effects of copper ions on DNA binding and cytotoxic activity of a chiral salicylidene Schiff base. J. Photochem. Photobiol., B, 2014; 132 : 36–44.
4. Marzano C, Pellei M, Tisato F, Santini C. Copper complexes as anticancer agents. Anticancer Agents Med. Chem, 2009; 9: 185–211.
5. Ferraria MB, Bisceglie F, Buschini A, Franzoni S, Pelosi G, Pinelli SP, Tarasconi P, Tavone M. Synthesis, structural characterization and antiproliferative and toxic bioactivities of copper(II) and nickel(II) citronellal N4-ethylmorpholine thiosemicarbazonates. J. Inorg. Biochem, 2010; 104: 199.
6. Krekoff I.H.,"Platinum and other metal coordination compounds in cancerchemotherapy:clinical application of platinum complexes", Nicoline, M. ED. Martinus Nijhoff Publishing: Boston, MA, P. 3511988 (1988).
7. Loehrer P. J., Williams S. D., Einhorn L. H., J. Natt. Cancer Inst., 80, 1373, (1988).
8. Loehrer P. J., Einhorn L. H., Ann. Intern. Med., 100, 704, (1984).
9. Krakoff I. H., Cancer Treat. Resp., 63, 1623, (1979).
10. Vermorken J. B., Pinedos H.M., Neth. J. Med., 25, 270, (1982).
11. Vonhoff D. D., Schilky R., Reichert C. M., Cancer Treat. Resp., 63, 1527, (1979).
12. Calvert A. H., Harland S. J., Newell D. R., Siddik Z. H., Jones A. C., McElwain T. J., Raju S., Wiltshaw E., Smith I. E., Baker J. M., Peckham M. J., Harrap K. R., Cancer Chemother. Pharmaco., 9, 140, (1982).
13. Harland S. J., Smith I. E., Smith N., Alison D. L., Platinum Coordination Complexes in Cancer Chemotherapy, Hacker M. P., Douple E. B., Krakoff I. H., Eds, Martinus Nihoff Publishing: Boston, MA, 352, (1984).
14. Egorin M. J., Van Echo D. A., Olman E. A., Whitacre M. Y., Forrest A. Aisner, J. Cancer Res., *45*, 650, (1985).
15. Harland S. J., Newell D. R., Siddik Z. H., Chadwick R., Calvert A. H., Harrap K. R., Cancer Res., 44, 1693, (1984).
16. Mauldin S. K., Husain I., Sancar A., Chaney S. G., Cancer Res., 46, 2876, (1986).
17. Fontes A. P. S., Almeida S. G., Nader L. A., Quím.Nova, 20, 398, (1997).
18. Beaumont K. P., McAuliffe C. A., Cleare M. J., Chem. Biol. Interact., 14, 179, (1976).
19. Paul A. K., Srivastava T. S., Chavan S. J., Chitnis M. P., Desai S., Rao K. K., J. Inorg. Biochem., 61, 179, (1996).
20. Farrell N. P., "Transition Metal Complexes as Drugs and Chemotherapeutic Agents", Kluwer Academic Publishers: Dordrecht, p. 18, (1989).
21. Monchak M., Goreshuk E., Davydov V., Mys'kiv M., "Synthesis and structure of copper complexes with N,N,N,'N'-penta allyl ethylenediammonium: $[(C_3H_5)_3NC_2H_4N(H^+)(C_3H_5)_2]$ $CuIICl_4$ and $[(C_3H_5)_3NC_2H_4N(H^+)(C_3H_5)_2]$ $CuIBr_3$", Chem. Met.Alloys, 2, 138-145, (2009).
22. Watkins C. L., Vigee G. S., "An investigation of ethylenediamine complexes of copper (II) and Nickel (II) in solution of Dimethylsulfoxide", J. Phy.Chem., 80(1), 83-88, (1976).
23. Watters J.I. and Loughman E. D., "Spectrophotometric investigation of mixed complex ion formed by copper (II) ion with pyrophosphate and ethylenediamine", J. Am Chem. Soc., 75(19), 4819-4823, (1953).
24. Basolo F., Chen Y.T. and Murmann R. K., "Chelating tendencies of C-substituted ethylenediamines of copper(II) and nickel ions", J. Am Chem. Soc., 76(4), 956-959,(1954).
25. Ukpong E.J., Prasad J., "Effect of counter ion, solvent and ligand substituents on reduction potentials of copper (II) complexes of various imidazoles in some nonaqueous solvents", Global Journal of Pure and Applied Sciences, 13(4), 533-536, (2007).
26. Hutchin S.M., Chapman K. T., Tetrahedron Lett. 35, 4055, (1994).
27. Lam P.Y.S., Jadhav P. K., Eyermann C. J., Hodge C. N., Ru Y., Bacheler L. T., Meck J.L., Otto M. J., Ragner M. M., Wong Y. N., Chang C. H., Weber P.C., Jackson D. A., Sharpe T. R., Viitanen E. S., Science, 263, 380, (1994).
28. Alexandrova R., Rasshkova G., Alexandrov I., Tsenova W., Tudose R., Costisor O., Experim. Pathol. and Parasitol., 1311, 6851, (2003).
29. Malmström B.G., Leckner J., Curr. Opin. Chem. Biol., 2, 286, (1998).
30. Robert M., Motekaits R. J., Martell A. E., Inorg. Chim. Acta, 103, 73, (1985).
31. Hutchin S.M., Chapman K. T., Tetrahedron Lett. 35, 4055, (1994).
32. Lam P.Y.S., Jadhav P. K., Eyermann C. J., Hodge C. N., Ru Y., Bacheler L. T., Meck J.L., Otto M. J., Ragner M. M., Wong Y. N., Chang C. H., Weber P.C., Jackson D. A., Sharpe T. R., Viitanen E. S., Science, 263, 380, (1994).
33. Chorev M., Goorman M., Acc. Chem. Res., 26, 266, (1993).
34. Zhao X., Chang Y. L., Fowler F. W., Lauher J. W., J. Am. Chem. Soc., 112, 6627, (1990).
35. Ibrahim G., Bouet G. M., Hall I. H., Khan M. A., J. Inorg. Biochem., 81, 29, (2000).
36. Dimmock J.R., Baker G. B., Epilepsy, 35, 648, (1994).
37. Christernsen J. J., Eatough D. J., Izatt R. M., Chem. Rev., (74), 351-384, (1974). (2) Nelson S. M., Pure Appl. Chem., 52, 2461-2476, (1980).