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Antioxidant and Catalytic Activity of Effective Curcumin Based Copper Complexes of 2-aminobenzothiazole Derivatives

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Abstract

A new series of Cu (II) complexes of curcumin compound have been synthesized from the bidentate schiff base ligands of 2-aminobenzothiazole of curcumin compounds. They were characterized by elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, UV-Vis., molar conductance, magnetic moment, and electrochemical studies. The binding behavior of the complexes with calf thymus DNA has been investigated by electronic absorption and cyclic voltammetry techniques. It reveals that these complexes interact with CT DNA through partial intercalation binding mode. The antioxidant activity of the ligands and their complexes were determined by the superoxide dismutase activity and hydrogen peroxide scavenging methods. A structural analysis of the tested copper complexes suggests that OH substitution and conjugation are important determinants of the free radical scavenging activity and electrochemical behavior. The electron-withdrawing substituent on the intercalative ligand can improve the DNA interaction affinity, free radical scavenging activity whereas the electron-donating substituent exhibits lesser activity. It is found that the DNA interaction affinity, free radical scavenging activity and antibacterial activity of the complex can be effectively controlled by the substituent.

Keywords: Knoevenagel condensate, DNA Binding, Antimicrobial activity.

INTRODUCTION

Neurodegenerative disease like parkinson's disease (PD), alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS) is due to the oxidative stress [1]. Effective natural antioxidants are epigallocatechin 3-gallate (EGCG), quercetin, resveratrol, carnosicacid, and rosmarinic acid, curcumin have each been shown to be effective scavengers of free radicals and could serve as the novel and safe therapeutic options for these devastating disorders [2]. Curcumin is a traditional herb obtained from curcuma longa, used for the treatment of cancer (leukemia, colon, liver, breast and prostate), alzheimer's disease, HIV, chronic inflammations, oxidative stress, anti-oxidant [3]. The isolation of Curcumin from Curcuma Longa is difficult, due to its low bioavailability and its instability at neutral to basic conditions that limit the development of curcumin as a potential therapeutic agent [4].

Curcumin compounds synthetically prepared by aldol condensation of aldehyde with terminal alpha carbon atoms of the enolic alkyl β-diketone lead to the formation of curcumin related compound. Knoevenagal condensation is a widely employed method for the synthesis of β-diketone [5]. Antioxidants are therefore becoming increasingly important as potential disease prevention and therapeutic agents. Since oxidative stress is a multi-stressor agents combining a range of different antioxidant properties, such as redox catalysis and metal binding, might be more effective and selective than mono-functional agents.

Benzothiazole derivatives of benzophenones containing 1,3-thiazol moiety show important antioxidant activity and low cytotoxicity and could decrease reactive oxygen species production generated by tert-butyl hydroperoxide (tBHP) [6]. Transistion metals are incorporated as functional redox centres in antioxidant enzymes such as catalase and superoxide dismutase. Therefore, these metals are often classified as antioxidants nutrients [7]. The success of Cu complexes as potential therapeutics will most likely be due to their ability to increase SOD activity, leading to relief of oxidative stress in the generation of free radicals. The redox nature allows it to participate in catalytic chemistry, making it a suitable cofactor for a diverse range of enzymatic reaction. In addition they exhibit high affinity towards DNA binding. The deoxyribonucleic acid (DNA) is the main target molecule for most anticancer and antiviral therapies. Investigations on the interaction of DNA with small molecules are important in the design of new types of pharmaceutical molecules [8]. Several studies have been carried out on copper (II) complexes complexes to act as catalase-like activity.

A new series of copper complexes of curcumin compound from the schiff base ligands were synthesized by the condensation of Knoevenagal condensate acetoacetanilide followed by aldol condensation with same substituted aldehydes. The synthesized ligand system is highly conjugated like curcumin and has pronounced biological and pharmacological activities.

METHODS

Material

All chemicals and solvents used were analytical grade reagent and were purchased from Merck. The supporting electrolyte solution was prepared using analytical grade reagents and doubly distilled water. Calf thymus DNA purchased from Genei Bio laboratory, Bangalore, India.

Instrumentation

Elemental analysis of ligands and their copper complexes were carried out using Perkin-Elmer elemental analyzer. Molar conductance of the complexes was measured using a coronation digital conductivity meter. The ^{1}H NMR spectra of the ligands were recorded using TMS as internal standard. The chemical shifts are expressed in units of parts per million relative to TMS. The IR spectra of the ligands and their copper complexes were recorded on a Perkin-Elmer 783 spectrophotometer in 4000-200 cm^{-1} range using KBr disc. Electronic spectra were recorded in a Systronics 2201 Double beam UV-Vis., spectrophotometer within the range of 200-800 nm regions. Magnetic moments were measured by Guoy method and corrected for diamagnetism of the component using Pascal's constants. Cyclic voltammetry electrode was performed on a CHI 604D electrochemical analyzer with three system of glassy carbon as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as the reference electrode. Tetrabutyl ammonium perchlorate (TBAP) was used as the supporting electrolyte. The interactions between metal complexes and DNA were studied using electrochemical and electronic absorption techniques.

Synthesis of complexes:

The β-ketoanilides derivatives were synthesized by addition of equimolar quantity of hot ethanolic solution of aromatic aldehyde(s), such as L^1/3-hydroxy-4-methoxybenzaldehyde, L^2/3-hydroxy benzaldehyde, L^3/3-chlorobenzaldehyde, L^4/4-methoxy benzaldehyde, L^5/4-nitrobenzaldehyde, L^6/4-dimethylamino benzaldehyde, L^7/3-nitrobenzaldehyde, L^8/3-phenylpropenal, L^9/2-chlorobenzaldehyde,and L^{10}/3,4-dimethoxybenzaldehyde to acetoacetanilide. The reaction mixture was stirred for about 2 hrs before refluxed at 60°C for the appropriate time using potassium carbonate as the catalyst. The precipitate obtained was dissolved in 50 ml dichloromethane, kept under reduced pressure and recrystallization from pure ethanol to lead to the formation of yellow colored diketone.

Aldol condensation proceeds with same aldehyde with the terminal alpha carbon atoms of the alkyl diketone to the formation of curcumin analog compound. The reaction was proceeded with equimolar quantity of adding hot ethanolic solution of β-ketoanilide(s) with corresponding aromatic aldehyde(s) such as L^1/3-hydroxy-4-methoxy benzaldehyde, L^2/3-hydroxy benzaldehyde, L^3/3-chloro benzaldehyde, L^4/4-methoxy benzaldehyde, L^5/4-nitro benzaldehyde, L^6/4-dimethylamino benzaldehyde, L7/3-nitrobenzaldehyde, L^8/3-phenylpropenal, L^9/2-chloro benzaldehyde, and L^{10}/3,4-dimethoxy benzaldehyde in the presence primary amine with continuous stirring for about 2 hrs and further undergo refluxed about 8 hrs.

Curcumin compound (1 M) was dissolved in ethanol and was added to 2-amino benzothiazole (2 M) with stirring at room temperature. The resulting solution was refluxed about 8 hrs at 45°C and the product formed was poured into ice kept at refrigerator for about 24 hrs. An ethanolic solution of schiff base (1 M) was mixed with copper chloride (1 M) in ethanol solution with continuous stirring. The mixture was then refluxed for 7 hrs till the volume of the solution was reduced to 10 mL. The complexes were precipitated in dry diethylether. The solid product obtained was filtered, washed with distilled water and cold ethanol and then dried in vacuum to afford brown and green colored precipitates.

DNA Binding Studies

The complexes were dissolved in DMSO and then diluted to the desired concentration with Tris-HCl buffer. The complexes remained dissolved after dilution. The spectroscopic titrations were carried out by adding increasing amounts of CT DNA to a solution of the complex at a fixed concentration contained in a quartz cell and recording the UV-Vis spectra after each addition. Solutions of CT DNA (calf-thymus DNA) in 50 mM NaCl/50 mM tris-HCl (pH = 7·2) gave a ratio of UV absorbance at 260 and 280 nm, A260/A280 of ~1·8–1·9, indicating that DNA was sufficiently free of protein contamination [9]. The concentration of DNA was determined by UV absorbance at 260 nm after 1: 100 dilutions. The molar absorption coefficient was taken as 6600 M^{-1} cm^{-1}.

Catalase-like activity studies

The decomposition of H_2O_2 by Cu(II) complex can be monitored by titrating the undecomposed H_2O_2 with standard $KMnO_4$ solution (0.01 M). 50 mg Cu(II) complex (0.1 mmol Cu) loaded in a flask containing a 10 ml of 3.5 $\times 10^{-2}$M hydrogen peroxide solution in aqueous phosphate buffer pH 6.86. Then, the reaction flask was thermostatted to 25°C under constant stirring. Finally, the extent of hydrogen peroxide decomposed at different intervals of time was estimated by taking 2.5 ml aliquot of reaction mixture and titrating it with 0.01 M $KMnO_4$ in the presence of 0.01 M H_2SO_4. The procedure was repeated at different amount of Cu(II) complexes [10].

Antioxidant Assay

The superoxide dismutase activity (SOD) of the copper(II) complexes were evaluated using alkaline DMSO as source of superoxide radicals ($O_2^{\bullet-}$) generating system in association with nitro blue tetrazolium chloride (NBT) as a scavenger of superoxide. 2.1 ml of 0.2 M potassium phosphate buffer (8.6 pH) and 1 ml of 56 µL of NBT solutions are added to the different concentration of copper complex solution. The mixtures were kept in ice for 15 min and then 1.5 ml of alkaline DMSO solution was added while stirring. The

absorbance was monitored at 540 nm against a sample prepared under similar condition except NaOH was absent in DMSO [11]. A solution of hydrogen peroxide (2.0 mm) was prepared in phosphate buffer (0.2 M, 7.4 pH) and its concentration was determined spectrophotometrically from absorption at 230 nm. The complexes of different concentration and Vitamin C (100 µg/ml) were added to 3.4 ml of phosphate buffer together with hydrogen peroxide solution (0.6 ml). An identical reaction mixture without the sample was taken as negative control. The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against the blank (phosphate buffer).

Antimicrobial activities

The in vitro antimicrobial activities of the investigated compounds were tested against the bacterial species by Serial Dilution Method. 100 mg of the compounds dissolved in DMSO was transferred to each disc with the help of a micropipette; the plates were allowed to stand for an hour in order to facilitate the diffusion of the drug solution. Then the plates were incubated at 37°C for 24 hr for bacteria and the diameter of the inhibition zones was read. Minimum inhibitory concentrations (MICs) were determined by using disk diffusion method [12]. The lowest concentration (mg/mL) of compound, which inhibits the growth of bacteria after 24 hr incubation, was taken as the MIC.

RESULTS AND DISCUSSION

FT-IR Spectroscopy

The formation of complexes was confirmed by the presence of new characteristic vibrational bands in the spectra of complexes. The significant IR absorption bands of the prepared ligands and their copper (II) complexes are given in the Table-1. It is observed from the spectra that the disappearance of characteristic band for a C=O group indicates the formation of schiff base ligand system (C=N) of 2-amino benzothiazoles. The appearance of C=N bands in the region of 1648-1631 cm^{-1} indicates the completion of schiff base ligand system (C=N) of 2-amino benzothiazoles. The shifting of C=N stretching frequency to 1648–1606 cm^{-1} indicates the formation of coordination bonds between the metal and nitrogen atom of the imine group. This supports the participation of the imine group of the ligand in binding to the copper(II) ion. Accordingly, the ligand acts as a bidentate chelating agent, bonded to the metal ion via the two nitrogen (–C=N) atoms of the schiff base. New bands at 443-478 cm^{-1} could be attributed to ν(M–N) respectively [13-14]. The IR Spectrum of $[CuL^1Cl_2]$ shows two different –C=N bands in the region of 1626 cm^{-1} and 1606 cm^{-1} and it coordinate with the metal ion in the region of 446 cm^{-1} and 474 cm^{-1}. The two ν (M–Cl) bands appeared in 374 cm^{-1} and 352 cm^{-1}.

Table1: IR spectral data (cm^{-1}) for the free ligands and their copper complexes

Compound	ν(C=N) cm^{-1}	ν(C=N) cm^{-1}	ν(M-Cl) cm^{-1}	ν(M-N) cm^{-1}
L^1	1646	1632	-	-
L^2	1645	1631	-	-
L^3	1642	1636	-	-
L^4	1647	1634	-	-
L^5	1646	1633	-	-
L^6	1643	1637	-	-
L^7	1646	1636	-	-
L^8	1644	1639	-	-
L^9	1642	1638	-	-
L^{10}	1648	1634	-	-
$[CuL^1Cl_2]$	1626	1606	374, 352	474, 446
$[CuL^2Cl_2]$	1624	1608	378, 352	474, 446
$[CuL^3Cl_2]$	1628	1610	369, 354	478, 451
$[CuL^4Cl_2]$	1626	1608	364, 344	468, 453
$[CuL^5Cl_2]$	1623	1609	371, 350	472, 443
$[CuL^6Cl_2]$	1625	1611	381, 356	469, 452
$[CuL^7Cl_2]$	1623	1610	374, 352	472, 443
$[CuL^8Cl_2]$	1630	1613	377, 359	478, 456
$[CuL^9Cl_2]$	1628	1612	369, 354	478, 451
$[CuL^{10}Cl_2]$	1623	1612	375, 356	471, 457

Electronic Spectroscopy

The electronic spectral measurements were used for assigning the stereochemistries of metal ions in the complexes based on the positions and number of d–d transition peaks. Spectral features assigned for the complexes are given in Table 2. The electronic spectra of schiff base ligands of substituted 2-amino benzothiazole derivatives show strong bands in the range of 267-317 nm, which are attributed to n-π* and π-π* respectively. The complexes exhibit a very intense ligand based absorption band in the UV region of 279-319 nm and have been assigned to n-π transition suggesting involvement in coordination of the diimine nitrogen atoms. However, they exhibit only one strong intense broad band in the region of 407-490 nm assigned to d–d transitions corresponding to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition. This is characteristic of square planar environment around the copper (II) ion [15].

The electronic spectra of copper (II) complex of $[CuL^2Cl_2]$ showed two absorption bands at 318 nm and 432 nm. Though third transition arises in all other copper complexes, they are very close in energy and often appear in the form of small band envelope nearly in the visible region of 614–642 nm

which is typical of a distorted square based coordination geometry around copper(II). It is possible that the complexes undergo a structural change from trigonal bipyramidal to square based geometry. The observed intensities of the visible absorption spectra indicate that these transitions are d-d in nature and the energies are consistent with six or five or four coordinate copper complexes. It is found that the synthesized copper complexes have distorted square planar geometry.

Table 2: Electronic absorption spectral data of the copper complexes in DMSO solution

Compound	Solvent	Absorption (nm)	Band assignment	Geometry
L^1	DMSO	315	$\pi-\pi^*$	-
L^2	DMSO	315	$\pi-\pi^*$	-
L^3	DMSO	312	$\pi-\pi^*$	-
L^4	DMSO	317	$\pi-\pi^*$	-
L^5	DMSO	317	$\pi-\pi^*$	-
L^6	DMSO	313	$\pi-\pi^*$	-
L^7	DMSO	267	$n-\pi^*$	-
L^8	DMSO	316	$\pi-\pi^*$	-
L^9	DMSO	**312**	$\pi-\pi^*$	-
L^{10}	DMSO	316	$\pi-\pi^*$	-
CuL^1Cl_2]	DMSO	317 407	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar
[CuL^2Cl_2]	DMSO	318 432	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar
[CuL^3Cl_2]	DMSO	314	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar
[CuL^4Cl_2]	DMSO	318 447	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar
[CuL^5Cl_2]	DMSO	319 434	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar
[CuL^6Cl_2]	DMSO	316 490	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar
[CuL^7Cl_2]	DMSO	269 487	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar
[CuL^8Cl_2]	DMSO	319 478	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar
[CuL^9Cl_2]	DMSO	314	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar
[$CuL^{10}Cl_2$]	DMSO	320 490	$^2B_{1g}\rightarrow$ $^2A_{1g}$	Distorted Square planar

Absorption spectral titrations

UV–Visible spectroscopy has been performed to study the interaction of the complexes with DNA keeping both copper complexes and CT–DNA concentration constant as 25 µg/ml. The absorption spectra of (a) [CuL^1Cl_2], (b) [CuL^3Cl_2], (c) [CuL^5Cl_2] and (d) [CuL^7Cl_2] complex in the absence and presence of CT-DNA at various concentrations are shown in Fig.1. Table 3 shows the electrochemical parameters for the copper complexes on interaction with CT- DNA. The copper complex of [CuL^2Cl_2] exhibited a band at 318 nm and 432 nm in the absence of DNA as shown in Fig. 1b. The absorption bands of the complexes in the presence of DNA showed considerable red shift of 435 in the $\pi-\pi^*$ indicating the strong binding between copper complex with CT-DNA. The difference in binding affinity is due to the presence of ancillary ligands in the schiff base system. It shows that the compounds interact with CT-DNA by intercalating into the DNA base pairs. It observed from the results of electronic absorption spectroscopy that the d-d transition for all complexes at 435 nm indicates that the copper complexes interact with N of guanine of CT-DNA.

The introduction of substituted groups on the phenyl ring would be expected to hinder the partial intercalation of the phenyl ring and hence decrease the DNA binding affinity. The present complexes are involved in weaker intercalative interaction obviously due to the steric effect between the ligand and DNA double helix. The strongest binding affinity exhibited by the complex is expected on the basis of the additional aromatic ring of aldol condensation which enhances the extent of stacking of the diimine with the DNA base pairs. It is concluded that the free ligand and the copper complex can interact with CT-DNA through the partial intercalation mode of binding. This may be due to the copper complex with the electric effect and steric hindrance resulting in the relatively close stacking between the Cu(II) complex and the DNA base pairs [16].

DNA binding experimental studies:

The redox properties of all copper complexes in the absence of DNA were investigated by cyclic voltammetry in DMSO solution. Fig.2 shows the cyclic voltammogram of (a) [CuL^1Cl_2], (b) [CuL^3Cl_2], (c) [CuL^5Cl_2] and (d) [CuL^7Cl_2] complex in the absence and presence of CT-DNA concentration. As the potential is swept from 0.10 to -1.00, the reduction of Cu(II) to Cu(I) proceeds through three distinct processes initially ligand oxidation takes place with E_{pa} at 0.114 V and it display a quasi-reversible reduction of Cu(II)/Cu(I) couple at $E_{pc}1$=-0.330 V and $E_{pa}1$=-0.182 V with peak to peak separationof 148 mV/s scan rate, followed by a non-reversible reduction at E_{pc}=-0.941V. Cyclic voltammogram of [CuL^2Cl_2] showed two segments of cathodic and anodic peaks (Fig. 2b). The first segment, cathodic and anodic peaks were observed at $E_{pc}1$ = -0.893 V

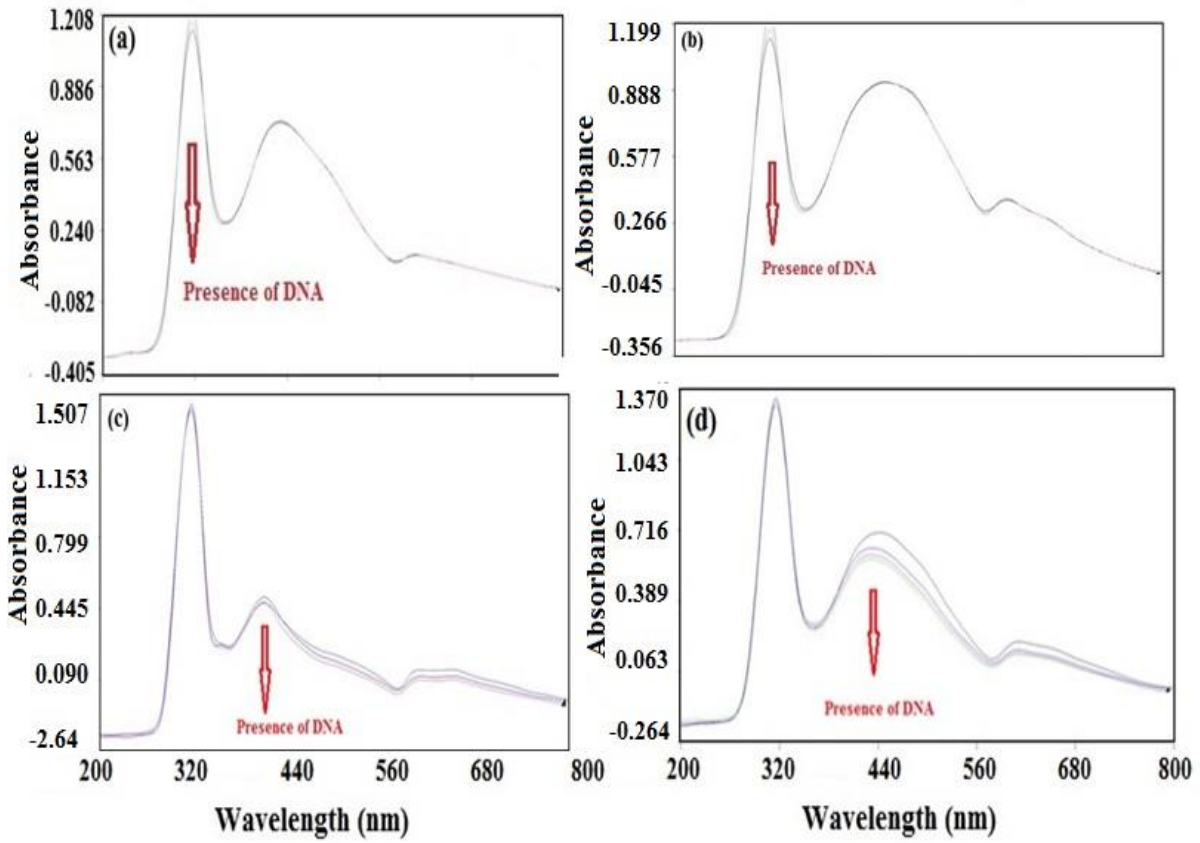

Figure 1: The absorption spectra of (a) $[CuL^1Cl_2]$, (b) $[CuL^3Cl_2]$, (c) $[CuL^5Cl_2]$ and (d) $[CuL^7Cl_2]$ complex in the absence andpresence of CT-DNA at various concentrations

and E_{pa1} = -0.381 V, respectively. This showed oxidation from +1 to +2 forms at a cathodic peak potential which corresponds to ligand oxidation and reduction behaviour, respectively. This showed unusual oxidation state of ligand system. The second segments of cathodic peaks and anodic peaks $E_{pc}2$ and $E_{pa}2$ were observed at -0.531 V and -0.210 V

Table 3: Electrochemical parameters for the copper complexes on interaction with CT- DNA

Compound	Redox couple	$E_{1/2}$(V)		ΔEp(V)		i_{pa}/i_{pc}
		Free	Bound	Free	Bound	
$[CuL^1Cl_2]$	Cu(II) →Cu(I)	-0.256	-0.660	-0.148	-0.130	1.10
$[CuL^2Cl_2]$	Cu(II) →Cu(I) Cu(I) →Cu(II)	-0.617	-0.566	-0.512	-0.502	1.21
$[CuL^3Cl_2]$	Cu(II) →Cu(I)	-1.015	-0.915	-0.610	-0.590	1.32
$[CuL^4Cl_2]$	Cu(II) →Cu(I)	-0.967	-0.952	-0.691	-0.684	1.11
$[CuL^5Cl_2]$	Cu(I) →Cu(II)	-0.123	-0.115	-0.103	-0.131	1.25
$[CuL^6Cl_2]$	Cu(II) →Cu(I)	-0.850	-0.801	-0.259	-0.239	1.10
$[CuL^7Cl_2]$	Cu(I) →Cu(II)	-0.260	-0.353	-0.341	-0.402	1.21
$[CuL^8Cl_2]$	Cu(II) →Cu(I)	-0.703	-0.646	-0.438	-0.450	1.23
$[CuL^9Cl_2]$	Cu(II) →Cu(I)	-0.935	-0.965	-0.561	-0.599	1.14
$[CuL^{10}Cl_2]$	Cu(I) →Cu(II)	-0.966	-0.957	-0.698	-0.686	1.12

respectively. This showed reduction occurs from +2 to +1 form at a cathodic peak potential.

The cyclic voltammograms of all other complexes display a quasi-reversible reduction at 130 - 684 mV with peak to peak separation value. This implies that peak potential increases with higher scan rates. The electrode processes are consistent with the quasi-reversibility of Cu(II)/Cu(I) couple [17]. This couple is found to be quasi-reversible as the peak separation between the anodic and cathodic potential is very high. The ratio between the anodic and cathodic currents

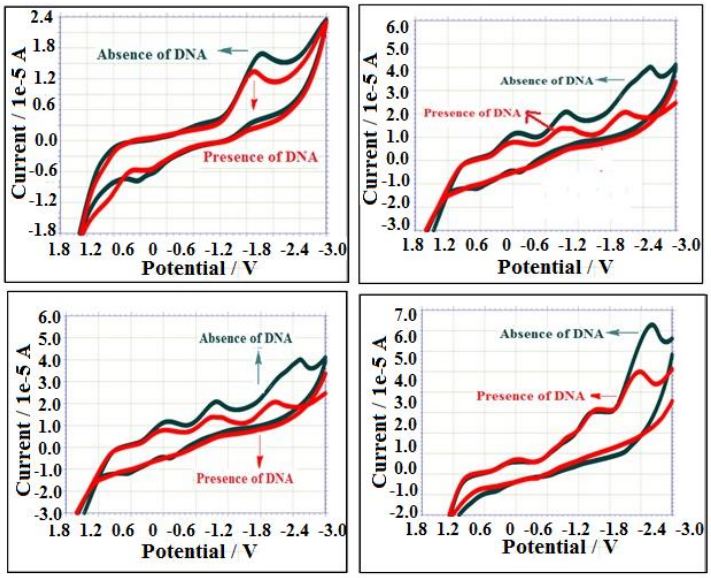

Figure 2: The cyclic voltammogram of (a) $[CuL^1Cl_2]$, (b) $[CuL^3Cl_2]$, (c) $[CuL^5Cl_2]$ and (d) $[CuL^7Cl_2]$ complex in the absence and presence of CT-DNA concentration.

suggests that the process is simple one-electron transfer, quasi-reversible process. Introduction of methoxy group shifts the E_{pa} towards more positive value and increases scan rates of $[CuL^4Cl_2]$ and $[CuL^{10}Cl_2]$. The peak potentials E_{pc} and E_{pa} in presence of DNA shifted towards positive values. It was proposed that the synthesized copper complexes intercalates into the base pairs of DNA. Moreover, the positive shifts of peak potentials also indicate the interaction between $[CuLCl_2]$ and DNA.

Cyclic voltammogram of $[CuL^3Cl_2]$ in the presence of DNA showed two segments of cathodic and anodic peaks as shown in Fig. 2b. The first segment, cathodic and anodic peaks were shifted to positive values towards Ep_{c1} = -0.210 V and Ep_a1 = -0.110 V, respectively. The same changes occurred at second segments of cathodic peaks and anodic peaks $E_{pc}1$ and $E_{pa}1$ at -0.814 V and -0.312 V, respectively. It shows that the strong affinity of $[CuLCl_2]$ and DNA might be caused by the coordination of cupric ion in $[CuL^2Cl_2]$ with guanine bases of DNA [18].

Thermal denaturation

Thermal behavior of CT-DNA in the presence of complexes gave insight into their conformational changes and the interaction strength of the complexes with DNA as the temperature is increased. The double- stranded DNA tends to gradually dissociate to single strands on increase in the solution temperature and generates a hyperchromic effect on the absorption spectra of DNA bases at 316 nm. The melting temperature (T_m) of DNA in the absence of copper complexes was found to be 54 ± 1°C. The addition of complexes increased the melting temperature T_m (± 1°C) from 10°C to 10.3°C for all copper complexes. This experimental data indicates that all the copper complexes of peptides have interaction with double helix CT-DNA. The significant increase of T_m (ΔT_m = 10.3ºC) suggests that the interaction of all the copper complexes with DNA is performed through partial intercalation shown in the Fig.3.

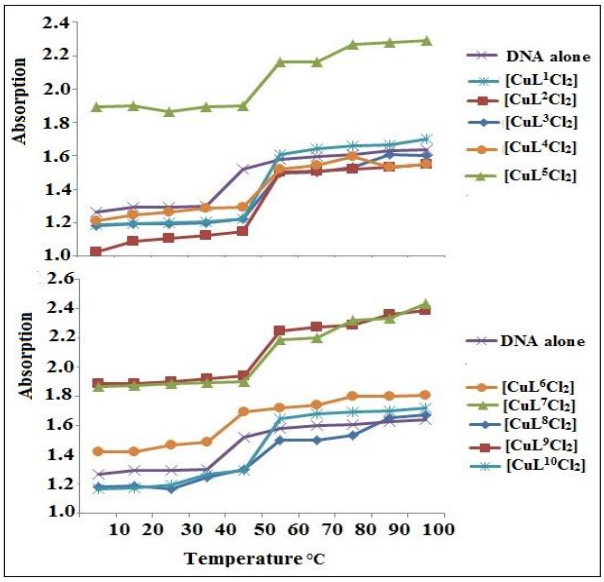

Figure3: Melting curves of CT-DNA in the absence and presence of copper complexes.

Antioxidant

The redox potential of almost all the complexes falls between 0 V to -1.6 V. It is found that compounds with strong scavenging capabilities are oxidized at relatively low potentials. The results reveal that the redox potential values of these complexes fall into the redox potential range that

Table 4: Antioxidant activity of schiff base copper complexes in (μmol dm^{-3})

Compound	IC_{50}(μmol dm^{-3}) OH^-	IC_{50}(μmol dm^{-3}) O_2•−
$[CuL^1Cl_2]$	55	53
$[CuL^2Cl_2]$	53	50
$[CuL^3Cl_2]$	85	79
$[CuL^4Cl_2]$	62	58
$[CuL^5Cl_2]$	76	71
$[CuL^6Cl_2]$	90	84
$[CuL^7Cl_2]$	74	69
$[CuL^8Cl_2]$	69	64
$[CuL^9Cl_2]$	82	77
$[CuL^{10}Cl_2]$	65	61
Sodium ascorbate	14.2	14.2
Bovin Erythrocyte	2.1	2.1

resembles the SOD enzyme [19]. The SOD Values of copper complexes were listed in Table 4. The synthesized copper

complexes have higher antioxidant activity due to the presence of highly conjugated curcumin analog system containing two azomethine groups. The redox properties of metal can serve as the structural models as well as good functional models of the enzyme that can decompose superoxide. The copper complexes [CuL^2Cl_2] exhibit excellent SOD mimic activity due to the presence of hydroxyl group that enhances enhanced the lipid peroxidation and oxidative damage to proteins. The complexes with nitro groups showed much lower anti-oxidant activity when compared to [CuL^2Cl_2]. All the tested compounds show SOD activity. Similar values obtained for all compounds. The antioxidant activity of synthesized complexes is in the order of :

[CuL^2Cl_2]>[CuL^1Cl_2]>[CuL^4Cl_2]>[$CuL^{10}Cl_2$]>[CuL^8Cl_2]>[CuL^7Cl_2]> [CuL^5Cl_2]>[CuL^9Cl_2]>[CuL^3Cl_2]>[CuL^6Cl_2]

Catalase like Activity

All the complexes showed the catalytic decomposition of H_2O_2. The catalytic activity of the complexes [CuL^1Cl_2], [CuL^2Cl_2], [CuL^4Cl_2], [CuL^8Cl_2] shows the best towards H_2O_2 decomposition. The time course of the O_2 evolution is shown in Fig. 4. The H_2O_2 disproportionation efficiency of the complexes follows the order: [CuL^8Cl_2]>[CuL^2Cl_2]>[CuL^1Cl_2]>[CuL^4Cl_2]. On the other hand, heterocyclic bases cause only a very slight disproportionation of the peroxide. The synthesized ligand system contains thiazole moiety responsible for increases their catalytic activities. This may demonstrate the importance of the heterocyclic bases that are known to exist in the vicinity of the active sites of the enzymes. Initially the decomposition takes place at slower rate and later it becomes faster. In the catalytic process, the electron transfer occurs only between the Cu(II) and Cu(II) ions in the dimer structure. Moreover, the presence of the bidentate chelating nitrogen donor ligand in the coordination sphere of the metal significantly enhances the ability of the copper to disproportionate H_2O_2 [20].

Antimicrobial activity

The *in vitro* antimicrobial activities of the investigated complexes were tested against the bacterial species, *Staphylococcus aureus, Escherichia coli, Klebsiellapneumaniae, Proteus vulgaris*, and *Pseudomonas aeruginosa* by Serial Dilution method. The inhibitions around the antibiotic discs were measured after incubation and Strephtomycin was used as standard drug. It was stated that the synthesized copper complexes of 2-amino benzothiazole derivatives showed more activity than its free ligands. It has also been suggested that the ligands bidentate nitrogen donor systems might inhibit enzyme production and show higher antimicrobial efficiency towards complexes than their ligands system. The inhibition activity seems to be governed by the facility of coordination at the metal centre as well as bulkiness of the ligands.

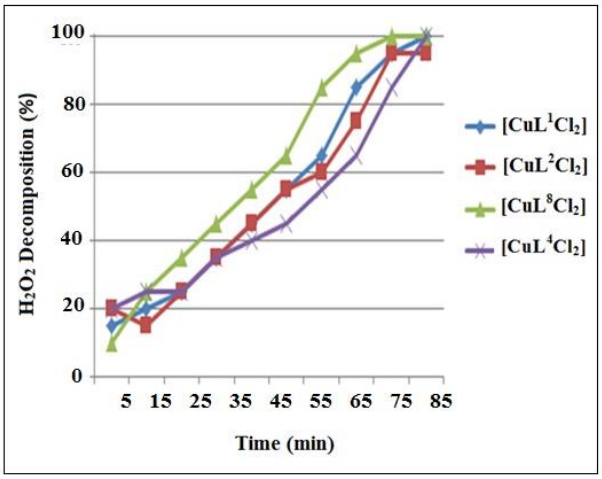

Figure 4: The time course of the O_2 evolution.

All the complexes tested revealed moderate to strong antimicrobial activity. Among all the test complexes attempted, [CuL^5Cl_2] and [CuL^7Cl_2] showed slightly higher activities against most gram positive than gram negative bacteria. All complexes show strong activity on the yeast cultures when compared with standard drug streptomycin. The antimicrobial activity of above copper complexes against microorganisms results in decrease in number of colonies (70-280). The complexes such as [CuL^5Cl_2] and [CuL^7Cl_2] gave lesser number of colonies. All the metal complexes are found to have higher antibacterial activity against Schiff base ligands [21]. The antibacterial results show that the activity of the schiff base compounds becomes more pronounced when coordinated to the metal ions. It was observed that increased activity was found in the order of

[CuL^5Cl_2]>[CuL^7Cl_2]>[CuL^9Cl_2]>[CuL^3Cl_2]>[CuL^8Cl_2]>[CuL^6Cl_2]> [$CuL^{10}Cl_2$]>[CuL^4Cl_2] [CuL^1Cl_2]>[CuL^2Cl_2].

CONCLUSION

The results reveal that the redox potential values of these complexes fall into the redox potential range that resembles the SOD enzyme. The synthesized copper complexes have higher antioxidant activity due to the presence of highly conjugated curcumin containing two imine groups and redox properties of metal can serve as the structural models as well as good functional models of the enzyme that can decompose superoxide. The copper complex of curcumin also has been found to exhibit antioxidant, superoxide-scavenging, and SOD enzyme-mimicking activities superior to those of ligands itself. The synthesized ligands and their copper complexes were fit into the antimicrobial and DNA binding. It is also shown that the synthesized copper complexes intercalate more preferentially to DNA than that of curcumin.

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