Newly Synthesized Copper (II) Complexes with 2-(benzo[d/thiazol-2-ylthio]-N'-(2-hydroxybenzylidene) acetohydrazide (BTHBA) and 2-((1,3-diphenyl-1H-pyrazol-4-yl) methylene) hydrazinecarbothioamide (DPPMHC) and their DNA binding and Antioxidant Activities

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ABSTRACT

The novel Cu(II) metal complexes synthesized from two heterocyclic Schiff base ligands 2-(benzo[d/thiazol-2-ylthio]-N'-(2-hydroxybenzylidene)acetohydrazide (BTHBA) and 2-((1,3-diphenyl-1H-pyrazol-4-yl)methylene) hydrazinecarbothioamide (DPPMHC) have been described. Schiff base ligands and metal complexes are characterized by various spectroscopic techniques. The FTIR and UV-Vis analysis confirmed the Cu and ligand bonding where a decrease in UV absorbance for complexes compared with pure copper acetate showed the transitions. The conductance values are low but showing their electrolytic nature. DNA binding testing the complexes have shown good binding nature which reveals their anticancer nature. Complexes have also shown their antioxidant nature against DPPH which shows their medicinal importance.

Keywords: Metal, Ligand, DNA binding, Antioxidant.

1. INTRODUCTION

Currently the transition metal complexes with Schiff bases have attracted attention of researchers due to their key role in the coordination chemistry and medicinal applicability. Schiff-base ligands with heteroatoms such as N, O, and S show a broad biological activity and are of special interest because of the variety of ways in which they are interacted to transition metal ions. Schiff-bases have expanded much importance not only in medicinal fields but also in catalysis, biomimetic modeling designing molecular magnet molecules, liquid
crystals\textsuperscript{4–5}. Since, Schiff-bases containing complexes are very useful in the field of medicine and industry thus these are very important materials for chemists\textsuperscript{6–9}. The medicinal potential is due to their diverse biological, pharmacological, antitumor activities and their outstanding chelating features\textsuperscript{10}. In this regard, the pharmaceutical potential of transition metal complexes containing Schiff base ligands is an attractive topic in chemistry, owing to their greater activities comparing to non - Schiff base complexes\textsuperscript{11–15}. Due to their cell membrane permeability they express important physiological and pharmacological activities. Furthermore, Copper is an important transition metal and its coordination compounds display interesting binding properties with proteins and nucleic acids. The N – and O – containing Schiff base ligands and their complexes have become important due to their wide biological activity\textsuperscript{16–21}. In addition, many Schiff-base transition metal complexes are suggested as enzymic-models, for instance urease. Apart from this, transition metal complexes also show the ability to bind and cleave DNA inferring their potential in the field of cancer chemistry and others. Since DNA is the first target of any anticancer drug. Experimental studies related to DNA binding and cleavage were explored using a range of potent Cu(II) Schiff-base complexes\textsuperscript{22–29}. In this context, there has been an interesting fact towards the development anticancer compounds of transition metal complexes derived from Schiff base ligands. Copper complexes are found to be beneficial reagents for oxidative and hydrolytic cleavage pathways of DNA\textsuperscript{30–38}. The first bis(phen)copper(I) complex was found to be able to cleave DNA in an oxidative manner\textsuperscript{39}. Thus, several copper-based complexes studied for their DNA binding activity as intercalator having highly targeted chemotherapeutical applications\textsuperscript{40–42}. It is recently shown that copper(II) complexes having dipyridoquinoxaline or NSO-donor Schiff base ligands are efficient photo-cleavers of DNA. Apart from the DNA binding, the antioxidant property has also been proven the anticancer activity of the compounds, and several studies have been reported where the antioxidant property has been found responsible for their anticancer activity. Thereby, synthesis, DNA and free radical have been targeted to design novel antitumor agents with a low reactivity in the blood stream and in non-tumoral tissues\textsuperscript{43–47}. With this interest, the present work stems from our interest to explore the DNA cleavage and antioxidant activities of new copper(II) complexes having different heterocyclic ligands Schiff bases.

2. EXPERIMENTAL

2.1 Materials and methods

All chemicals were procured from Sigma Aldrich and used as received. For characterization UV/ Vis, IR, \textsuperscript{1}HNMR, MS and CHN elemental analyzer spectroscopic tools were used. IR (Bruker Atr) spectra were taken in a KBr palate. \textsuperscript{1}H NMR spectra were recorded in DMSO (NMR, 99.99%) with a Bruker-Biospin Avance-III 500 MHz FT-NMR spectrometer using TMS as an internal standard for chemical shifts. Mass spectra were obtained with an Agilent Q-TOF LC/MS with ESI+ mode with DMSO as the mobile phase. Elemental analysis was performed with a Euro vector instrument. UV/Vis spectra were recorded with a Spectro 2060 plus model UV/Vis spectrophotometer over 200–600 nm using a 1 cm path length cuvette
in DMSO at 1 X 10^{-3} \text{ M (molar). The conductance was measured with LABINDIA, PICO+}
model conductivity meter at 25^\circ \text{C for 1x10^{-3} to 5 X 10^{-3} M (molar) solution in DMSO at 1 X}
10^{-3} \text{ interval. Aqueous 0.1M (12.88 mS/cm), 0.01M (1.413 mS/cm) and 0.001M (147\mu \text{S/cm})}
KCl solutions were used for calibration standard for conductivity meter.

2.2 Synthesis

Scheme 1.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme1}
\caption{2-(benzothiazol-2-ylthio)-N'-(2-hydroxybenzylidene) acetoxydrazide, (HL) : (BTHBA)}
\end{figure}

Ligand HL that is BTHBA was synthesized stepwise as per reported method\textsuperscript{48-49}.

\textbf{Step:1. 2-Mercaptobenzothiazoyl thioethyle acetate.}

\text{Ethyl chloroacetate (0.01mole) and 2-mercaptobenzothiazole (0.01mole) was refluxed in presence of anhydrous K}_2\text{CO}_3 (0.5gm) in methanol for 15hrs. on waterbath. After completion of reaction, reaction mixture was poured in ice cold acidic water to obtain product. Reaction was monitored by TLC (Toluene: Acetone, 1:3) Yield 80%. M.P. 58 \degree \text{C.}

\textbf{Step:2. 2-(Benzo[d]thiazol-2-ylthio)acetohydrazide:}

\text{2-Mercaptobenzothiazoyl thioethyl acetate (0.01mole) and hydrazine hydrate (0.01Mole) in methanol was refluxed on waterbath for 3hrs. on cooling crystalline product was obtained. Which was recrystallised in ethanol and solvent was removed in vacuo. Yield 75%. M.P. 193 \degree \text{C.}}
Step:3. 2-(Benzo[d]thiazol-2-ythio)-N'- (2-hydroxybenzylidene) acetohydrazide: Ligand (HL1): BTHBA.

2-(benzo[d]thiazol-2-ythio)acetohydrazide (0.01mole) and equimolar benzaldehyde in methanol was refluxed on waterbath for 3hrs. On cooling white precipitation of ligand BTHBA was obtained. Product was recrystallised from ethanol. Yield 55%, M.P. 168-170°C. Anal. Calcd. C, 55.96; H, 3.82; N, 12.24; O, 9.32; S, 18.67%. Found: C, 55.06; H, 4.02; N, 11.94; O, 9.02; S, 18.17%.

IR (KBr): v (OH) 3222 cm⁻¹, v (CONH) 1680 cm⁻¹, v (Ph, C=C) 1454, 1426,1553 cm⁻¹, v (Ph,C-H) 3071cm⁻¹, v(C-S-C) 1237,1268,1219,1205, v(NCS) 1377,1358 cm⁻¹, v(N-C-S Thiazole ring) 752,779, cm⁻¹, v (CH=N) 1620,1608 cm⁻¹, v (-N-N) 1410, 1395 cm⁻¹,

¹H NMR: δH (500 MHz; DMSO-d₆; Me₄Si) 3.368 (2H, s, CH₂), 10.1 (1H, s, OH), 6.8 - 8.457 (8H, m, 2-Ph), 11.7 (1H, s, NH), 9.01 (1H, s, CH=N).

+ve ESI-MS: m/z 344.4 [M+1] (calc. for [C₁₆H₁₃N₃O₂S₂]=343.04).

Scheme 2

2-((1,3-Diphenyl-1H-pyrazol-4-yl)methylene)hydrazinecarbothioamide: (HL3): (DPPMHC)

Ligand HL3 that is DPPMHC was synthesized stepwise as per reported method⁵⁰-⁵³.

$$\text{C}_6\text{H}_8\text{NH}_2\text{NH}_2 + \text{C}_6\text{H}_5\text{COCH}_3 \xrightarrow{\text{Acetic acid}} \text{Ethanol} \rightarrow \text{Acetophenone phenyl hydrazone}$$

$$\text{L}_3\text{dipyridyl-1H-pyrazolo-1-carbaldheyde (DPPMHC)}$$
Acetophenone (0.01 mol) and phenylhydrazine hydrochloride (0.01 mol) in acetic acid (1 ml) was refluxed on waterbath for 1 hr. in ethanol (10 ml). The obtained solid acetophenone hydrazone intermediate was filtered, washed with ethanol (2 ml) to give off-white free flow solid. Yield: 90-96%.

DMF (1.16 ml, 0.015 mol) was taken in a round bottom flask, cooled to -5 °C using ice salt mixture and added POCl₃ (2.8 ml, 0.03 mol) in about 1.5 h at 0-5 °C. The acetophenone hydrazone intermediate (0.005 mol) was added at 0 °C and refluxed the reaction mass to 70°C for 3 h. After the completion of the reaction, the reaction mixture was cooled and poured into ice-chilled water and neutralized with 10% sodium bicarbonate solution and the white solid product 1,3-diphenyl-1H-pyrazole-4-carbaldehyde (DPPC) was stirred for one hour at room temperature and filtered. Further, it was washed with distilled water and recrystallized the crude material from methanol. Yield: 66-91%. M.P. 140 °C. uncorrected

IR: ν (CHO) 1674 cm⁻¹, 1768 cm⁻¹, ν (CH=N) 1633 cm⁻¹, 1531 cm⁻¹, 1514 cm⁻¹.

¹H NMR: δH (500 MHz; DMSO-d₆; Me₄Si), 8.2-8.3 (2H, s, NH₂), 9.1 (1H , s, CH=) 7.8 (1H , s, NH), 7.95(1H , s, C₅H),

+ve ESI- MS: m/z 323.12 [M+1] (calc. for [C₁₆H₁₄N₆S]=322.10).

Scheme 3

Synthesis of metal complex: L₁Cu: Cu (L₁)₂ : Cu (BTHBA)₂

Copper acetate (Cu(CH₃COO)₂) (0.01 mol) and ligand BTHBA (0.02 mol) in 1,4 dioxane were refluxed on water bath for 10 hrs. in the presence of sodium acetate into RBF and stirring was given upto 1000 rpm. On completion of the reaction precipitate was obtained which was filtered and washed with distilled water and alcohol several times. Then it was dried up to absolute dryness in vacuum oven. Yield: 60%.

IR (KBr): v (OH) 3200-3400 (broad band) cm\(^{-1}\), v (Ph, C=) 3028,1456,1426 cm\(^{-1}\), v (Ph, C-H) 3028,1456,1426 cm\(^{-1}\), v (C=S-C) 1220,1280 cm\(^{-1}\), v (NCS) 480 cm\(^{-1}\), v (C=N) 1375,1343 cm\(^{-1}\), (N-C-S Thiazole ring) 768,752 cm\(^{-1}\), v (-N-N) 1350, 1300 cm\(^{-1}\), v (CH=N) 1615,1600,1507 cm\(^{-1}\), v (Cu-N) 650, 510,490 cm\(^{-1}\), v (Cu-O) 510.

\(^1\text{H NMR:} \delta \text{H (500 MHz; DMSO-d}_6\text{; Me}_4\text{Si)} 2.53-3.58 \text{ (}4\text{H, s, CH}_2\text{), 10.049 (2H, broad s, OH), 7.3-8.3 (8H, m, 2-Ph), 8.304 (2H, s, CH=N).\)

UV/Vis = max (DMF)/nm 317, 391.

6. Synthesis of metal complex: L₃Cu: Cu (L₃)₂ : Cu(DPPMHC)₂

Scheme 4

Copper acetate (Cu(CH₃COO)₂) (0.01 mol) and ligand DPPMHC (0.02 mol) were refluxed in 1,4 dioxane on waterbath for 12 hrs. in the presence of sodium acetate into RBF and stirring was given upto 1000 rpm. On completion of the reaction precipitate was obtained which was filtered and washed with distilled water and alcohol several times. Then it was dried up to absolute dryness in vacuum oven. Yield: 65\%.

IR (KBr): ν (NH₂) 3300, 3380, 3450 cm⁻¹, (Ph, C=C) 3080 cm⁻¹, (Ph, C-H) 1500, 1550, 1620 cm⁻¹, v (C-S-C) 1200, 1250, 1270 cm⁻¹, v (NCS) 760, 770, 830, 682 cm⁻¹, v (C-N) 1375, 1343 cm⁻¹, v (C=S) 1275, 830 cm⁻¹, ν (-N-N) 1340 cm⁻¹, v (CH=N) 1560, 1590 cm⁻¹, v (Cu-N) 480, 500 cm⁻¹.

¹H NMR : δH (500 MHz; DMSO-d₆; Me₄Si), 7.9 (4H, s, NH₂), 8.05 & 8.6 (2H, s, C₅H), 8.05 & 8.6 (2H, s, CₛH), 7.3-7.8 (20H, m, Ph).

2.3 DNA binding activity

Solutions were prepared w/v with ± 0.01 mg using Mettler Toledo electronic Kern balance. Absorption spectra were recorded with Spectro 2060 plus model UV/Vis over 200-600 nm on 1 cm path length cuvette. The CT-DNA (Sigma) was used as received (analytical grade). Tris–HCl buffer (10 M, pH=7.2) prepared in Milli-Q water, was used for DNA stock solution, absorption titration. The DNA concentration was determined by absorption spectroscopy with molar absorptivity (6600 M⁻¹ cm⁻¹) at 260 nm. The CT-DNA in buffer gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9, indicating that the DNA was sufficiently free of protein. Absorption titrations in Tris-buffer were performed with 10, 30, 50, 70 and 90 μM to which the DNA stock solutions (50 μM) was added, (ri = [compound]/[DNA] = 0.2, 0.6, 1, 1.4, 1.8). Complex-DNA solutions were incubated at room temperature for 15 min before taking absorption spectra. For the measurements, the DNA concentration was kept constant and concentrations of complexes were varied from 10 to 90 μM with 20 μM interval.

2.4 Antioxidant activity

The free radical scavenging effect was determined using 2,2-diphenyl-1-picryl hydrazyle (DPPH) free radical where both the stock solutions of 1000 μM of the samples and 0.002% DPPH were prepared in DMSO + water (1:1). One mL of 0.002% DPPH solution was added in 1 mL of 50, 100, 150, 200, 250, 300, 350 and 400 μM of the complex solutions

separately to prepare the test samples. Both the components as reacting mixtures were thoroughly mixed by shaking the test tubes vigorously and incubated at 25°C by keeping in a water bath in dark for 60 min. An absorbance was measured at 517 nm with UV/Vis spectrophotometer. The interaction activity was identified in terms of a decrease in absorbance of DPPH and calculated with following formula:

Interaction activity % = \( \frac{A_0 - A_S}{A_0} \times 100 \)

A_S is an absorbance of DPPH with a tested compound and A_0 is of DPPH without a tested compound (control). The data calculated are presented as means ± SD of three determinations.

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization

Table 1. Conductivity analysis of the synthesized complexes in DMSO at room temperature.

<table>
<thead>
<tr>
<th>Conductance in DMSO (μS)</th>
<th>Cu(BTHBA)_2</th>
<th>Cu(DPPMHC)_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>14.6</td>
<td>16.6</td>
</tr>
<tr>
<td>0.002</td>
<td>20.5</td>
<td>20.9</td>
</tr>
<tr>
<td>0.003</td>
<td>24.8</td>
<td>25.8</td>
</tr>
<tr>
<td>0.004</td>
<td>29.1</td>
<td>30.1</td>
</tr>
<tr>
<td>0.005</td>
<td>32.9</td>
<td>33.6</td>
</tr>
</tbody>
</table>

The molar conductance values of all the complexes were determined in 10^{-3} M DMSO and are tabulated in Table 1. The conductivity values of Cu(II) complexes are in the range around 34 mhos cm2 mol^{-1} indicating the non-electrolytic nature of the complexes showing that these complexes have covalent behaviour. The observed very small conductance values show that practically no interaction occurs between the solvent and the dissolved complex.

Figure 1 and 2 depict the UV/Vis absorption for the pure Cu salt, ligand and Cu complexes with the ligands where the absorption for the pure Cu salts is much higher than the complexes confirming the formation of complexes. The UV/Vis absorption at 317, 391 (Figure 1 and 2) have confirmed the \( \pi-\pi^* \), L \( \rightarrow \) M (LMCT), 2B_{1g} \( \rightarrow \) 2A_{1g} transition where first band may be due to \( \pi-\pi^* \) transition within the aromatic ring and later band due to n-\( \pi^* \) transition within -C=N group. Upon complexation, n-\( \pi^* \) transition of ligand shifts to a longer wavelength; this indicates the coordination of ligand to metal\(^{54}\).

Figure 1. Absorption spectra of Cu(BTHBA)_2.
On the basis of IR spectra of ligand BTHBA and it’s metal complex shown in the table -1 with Cu(II) ion it shows that Cu(II) ion is bonded through oxygen of -C=O and nitrogen of – NH groups of the ligand. IR band at 1615, 1600 cm\(^{-1}\) in the complex indicates metal ion is not bonded through azomethine group (CH=N) of ligand. Band at 768, and 752 cm\(^{-1}\) indicates metal ion is not bonded through N or S of thiazole ring even. Disappearance of band at 1681 cm\(^{-1}\) in the complex indicates coordination through CONH group. Band in the IR region 469-470 and 430-470 cm\(^{-1}\)indicates presence of Cu-O and Cu-N bond formation. Absence of band at 1718-1730 cm\(^{-1}\) indicates absence of acetate ion in the complex.

Table-2 Comparison of IR spectra of ligand BTHBA and it’s metal complex.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\nu)- (OH) cm(^{-1})</th>
<th>(\nu)- (CH=N) cm(^{-1})</th>
<th>(\nu)- (CONH) cm(^{-1})</th>
<th>(\nu)- (N-C=S) cm(^{-1})</th>
<th>(\nu)- (Thiazole ring) cm(^{-1})</th>
<th>(\nu)- (Cu-O), (Cu-N) cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTHBA</td>
<td>3222</td>
<td>1620, 1608</td>
<td>1681</td>
<td>1377, 1358</td>
<td>1268, 1237, 1205</td>
<td>1570,1553, 752,779</td>
</tr>
<tr>
<td>Cu(BTHBA)(_2)</td>
<td>3200-3400 broad</td>
<td>1615, 1600</td>
<td>Nil</td>
<td>1375, 1343</td>
<td>1280, 1237, 1205</td>
<td>1570,1553, 752,768</td>
</tr>
</tbody>
</table>

Table-3 Comparison of IR spectra of ligand DPPMHC and it’s metal complex

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\nu)- (NH(_2)) cm(^{-1})</th>
<th>(\nu)- (CH=N) cm(^{-1})</th>
<th>(\nu)- (N-C=S) cm(^{-1})</th>
<th>(\nu)- (C=S) cm(^{-1})</th>
<th>(\nu)- (Thiazole ring) cm(^{-1})</th>
<th>(\nu)- (Cu-O), (Cu-N) cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPMHC</td>
<td>3150, 3250,3350</td>
<td>1625, 1650</td>
<td>1350</td>
<td>1275, 1350</td>
<td>1275,1340, 760,770, 830</td>
<td>830</td>
</tr>
<tr>
<td>Cu(DPPMHC)(_2)</td>
<td>3300-3450</td>
<td>1560, 1590</td>
<td>1340</td>
<td>1275,1340, 760,770, 830</td>
<td>830</td>
<td>480,500</td>
</tr>
</tbody>
</table>

On the basis of IR spectra of ligand DPPMHC and it’s metal complex shown in the table -2 with Cu(II) ion it shows that Cu(II) ion is bonded through nitrogen of -CH=N and nitrogen of –NH groups of the ligand. DPPMHC ligand shows characteristic IR band of \(\nu\) (NH\(_2\)), \(\nu\) (NH), at 3150, 3250, 3350 cm\(^{-1}\). The strong band at 1625,1650 cm\(^{-1}\) region assigned...
to azomethine (–CH=N) group. Presence of bands in the region 800-1500 indicates presence of presence of C=S stretching. Band at 1275, 830 cm\(^{-1}\) indicates presence of pure –C=S. Metal complex also shows presence of band at 3300-3450 cm\(^{-1}\) indicates presence of –NH\(_2\) group in the complex reveals that Cu(II) ion is not bonded through -NH\(_2\) group of the ligand. The band appeared around 1625-1650 cm\(^{-1}\) in the ligand is shifted to lower wave no. 1560-1590 cm\(^{-1}\) indicating that the azomethine (-CH=N) group of the ligand has coordinated to the Cu(II) metal ion through nitrogen. Absence of IR band at 1718-1730 cm\(^{-1}\) indicates absence of acetate ion in the complex. The characteristic bonding between Cu and nitrogen of ligands were obtained at 650, 500, 480 cm\(^{-1}\). In the case of complexes (schemes 3 and 4) the FTIR spectra has confirmed the formation of complexes that is supported by UV/Vis analysis.

The presence of protons in different environment was confirmed for ligand BTHBA with \(_1\)H-NMR where 2H of -CH\(_2\) appeared at 3.368 δ with singlet and 1H of -OH group appeared at 10.1 δ with singlet. Protons of phenyl ring were found in the range of 6.8-8.457 δ as given in characterization data. The protons of NH and CH= were appeared at 11.7 δ and 9.01 δ with singlet respectively in NMR spectra. Absence of NMR signal of –NH in complex at 11.7 δ value indicates Cu-N bonding while presence of signal at 10.049 δ value indicates –OH group intact and not taken part in the bonding with Cu(II) ion.

The presence of protons in different environment was confirmed for ligand DPPMHC with \(_1\)H-NMR where 2H of -NH\(_2\) appeared at 8.2-8.3 δ with singlet and 1H of -NH group appeared at 7.8 δ with singlet. 1H of -C\(_5\)H of pyrazole ring was found at 7.95 δ with singlet, 1H of azomethine (-CH=N) was found at 9.1 δ with singlet and 1H of aromatic nucleus found in the range 7.3-7.95 δ as multiplet given in characterization data.

NMR signals of the complex Cu(DPPMHC)\(_2\) at 7.9 δ with singlet indicates presence of two –NH\(_2\) groups in the complex reveals nitrogen of the amino group has not taken part in the complex formation. Similarly absence of signal of NH proton at 7.8 indicates nitrogen of CSNH has bonded with Cu(II) ion.

The mass spectra showed a mass 344.4 confirmed the mass of derived structure. The 323.12 mass was obtained in mass spectra confirming its structural mass of the ligands.

### 3.3 DNA binding activity

![Figure 3. Absorption spectra of DNA (5x10^-5M) in absence and presence of increasing amounts of Cu(BTHBA)\(_2\) and Cu(DPPMHC).](image)
For a concept of structure activity relationship (SAR) of the complex, the DNA binding is an effective mechanism. DNA binding shows the anticancer activity of complex because cancer can be prevented by the blocking the DNA of cancerous cell since uncontrolled DNA replication is responsible for the cancer. Thus, the synthesized Cu(BTHBA)$_2$ and Cu(DPPMHC)$_2$ complexes have been interacted with CT DNA to measure their DNA binding nature. Pure DNA shows a characteristic absorption band at 260 nm in UV spectra, if complex interacts with DNA than there changes are noted in absorption at 260 nm$^{58-61}$. Generally, hypochromism and hyperchromic effects are considered as intercalating strength of complexes with the bases of DNA. Figure 3 shows the DNA binding nature of Cu(BTHBA)$_2$ and Cu(DPPMHC)$_2$ complexes where a significant hypochromic effect was noticed expressing the intercalation between complexes and base pairs of DNA. For pure DNA the absorption at 260 nm was 0.33 a maximum but when it was interacted with complexes then there was a decrease in absorption at the same wavelength (Fig. 3). According to absorption analysis as shown in Figure 3, the Cu(BTHBA)$_2$ has shown high DNA binding affinity as compared to Cu(DPPMHC)$_2$. In such titration the intercalating affinity of complexes depend on size, electron density of interacting aromatic rings and number of binding cites available on ligand which are reinforced by the attachment of metal. Thereby, the complexes Cu(BTHBA)$_2$ and Cu(DPPMHC)$_2$ have shown their DNA interaction which leads them for anticancer analysis in vitro as well as in vivo.

3.4 Antioxidant activity

<table>
<thead>
<tr>
<th>Conc. of complex (µM) in 0.002% DPPH</th>
<th>Cu(BTHBA)$_2$ Absorption at 517 nm</th>
<th>Cu(DPPMHC)$_2$ Absorption at 517 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.231</td>
<td>0.295</td>
</tr>
<tr>
<td>100</td>
<td>0.227</td>
<td>0.308</td>
</tr>
<tr>
<td>150</td>
<td>0.226</td>
<td>0.311</td>
</tr>
<tr>
<td>200</td>
<td>0.230</td>
<td>0.311</td>
</tr>
<tr>
<td>250</td>
<td>0.240</td>
<td>0.324</td>
</tr>
<tr>
<td>300</td>
<td>0.257</td>
<td>0.307</td>
</tr>
<tr>
<td>350</td>
<td>0.269</td>
<td>0.324</td>
</tr>
<tr>
<td>400</td>
<td>0.268</td>
<td>0.335</td>
</tr>
<tr>
<td>Pure DPPH (0.002%)</td>
<td>0.456</td>
<td>0.456</td>
</tr>
</tbody>
</table>

The antioxidant activity of synthesized complexes Cu(BTHBA)$_2$ and Cu(DPPMHC)$_2$ is investigated on the basis of decrease in absorption of free radical DPPH. DPPH gives a maximum absorption at 517 nm in visible region of spectra, and when any chemical species traps it, the decrease in absorption is found at the same wavelength. The antioxidant nature of complexes is also found to be responsible for their anticancer nature up to certain extent. Therefore in this context, the antioxidant activity of Cu(BTHBA)$_2$ and Cu(DPPMHC)$_2$ is analyzed. Table 4 shows that pure DPPH has shown absorption 0.456 at 517 nm while with complexes having 50 to 400 µM at an interval of 50 µM has shown a decrease in absorption. A quantitative analysis of antioxidant activity was done with a concentration-dependent mode by comparing the absorption of DPPH with and without complexes at 517 nm, as shown in
figure 4. Cu(BTHBA)$_2$ has shown maximum %antioxidant activity 50.44% at 150μM while Cu(DPPMHC)$_2$ showed 35% at 50μM which inferred the high antioxidant nature of Cu(BTHBA)$_2$.

4. CONCLUSION

Synthesis, DNA binding and antioxidant activities of Cu(BTHBA)$_2$ and Cu(DPPMHC)$_2$ have been reported. The DNA binding nature of complexes has shown their applicability in cancer field where they can be treated as anticancer drug after in vitro and in vivo analysis. For proving their medicinal applications the antioxidant activity has also been determined where the complexes have exhibited an effective antioxidant activity, and the scavenging effect was noted from up to 50%. Thus, the DNA binding and antioxidant nature have confirmed their application in the field of medical.

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REFERENCES

50. Harikrishnaa,b N., Arun M. Islara, Ananda K.c, Abdulrahman Obaidd, and Hoong-KunFunde Published on 01 May 2015. Downloaded by Fudan University on 05/05/2015.