Synthesis, Characterization, Computational, Equilibrium, DNA cleavage and Antimicrobial Studies of Cu(II) and Zn(II) Complexes with 1-Acetyl-4-methyl-3-thiosemicarbazide

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ABSTRACT

Cu(II) and Zn(II) metal complexes with novel Schiff base 1-acetyl-4-methyl-3-thiosemicarbazide (AMTSC) were synthesized and characterized by LCMS, IR, $^1$H-NMR ($D_2O$ exchangeable), $^{13}$C-NMR, UV-Visible spectra, ESR, TGA, molar conductance and magnetic susceptibility measurements. The spectro-analytical studies reveal the composition of complexes as ML$_2$ for Cu(II)-AMTSC and ML for Zn(II)-AMTSC systems. The proton-ligand dissociation constant and metal-ligand formation constants of AMTSC with Zn(II) were determined in 70% (v/v) DMF-water medium at 0.1M (KNO$_3$) ionic strength and 303K using potentiometric Irving-Rossotti titration technique. AMTSC acts as monobasic ligand by releasing proton from amide via enol form and forms 1:1 (Zn-L) complex in solution. Computational energy calculations (semi-empirical) were performed on geometrically optimized thione-thiol and keto-enol forms of AMTSC to evaluate energy parameters and to generate surface energy maps. The cleavage of plasmid pBR322 DNA without any additives was monitored by gel electrophoresis and these complexes exhibited hydrolytic cleavage of plasmid DNA. The antibacterial activity of the compound and its complexes were tested against gram positive and gram negative bacteria.

Keywords: DNA cleavage, ESR, potentiometric titrations, semi-empirical.

INTRODUCTION

Thiosemicarbazides are biologically important compounds$^1$. Substituted thiosemicarbazides has been shown to possess a wide range of pharmacological properties such as antifungal$^{2,3}$,
antibacterial\textsuperscript{4,5}, antimalarial\textsuperscript{6}, antiinflammatory, anticonvulsant, antidepressant, antiviral, antitumor activity\textsuperscript{7-14}. Thiosemicarbazide group is believed to be carrier of therapeutic value and its derivatives possess biological activity because of their ability to bind to metal ions. The study of these compounds is interesting because of their tendency to form mononuclear and poly nuclear complexes depending on the nature of metal ion\textsuperscript{15}. The anti-microbial and anti-rheumatic activity\textsuperscript{16} of acyl-thiosemicarbazides created interest among researchers to study new thiosemicarbazide derivatives and their chelating properties.

The present work explains the synthesis, characterization, computational, equilibrium studies, DNA cleavage and antibacterial studies of novel compound 1-acetyl-4-methyl-3-thiosemicarbazide (AMTSC) and its metal complexes with Cu(II) and Zn(II) ions.

**EXPERIMENTAL**

**Materials and Methods**

All the chemicals and solvents used were of AnalR grade. Metal chloride salts [MCl\textsubscript{2} M= Cu(II) and Zn(II)] were used for the synthesis of complexes.

Conductivity measurements of the metal complexes were carried out in DMSO (1×10\textsuperscript{-3} M) using Digisun digital conductivity meter model D1 909. LCMS of all the compounds were recorded on LCMS 2010 A, Shimadzu spectrophotometer. \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR spectra were taken on Bruker 400mHz NMR spectrophotometer. Semi-empirical AM1 quantum chemical calculations were performed by the HyperChem\textsuperscript{TM} 7.5 Molecular Modeling program. IR spectra were recorded in KBr phase (4000cm\textsuperscript{-1} to 250cm\textsuperscript{-1}) on Schimadzu IR prestige-21 FTIR spectrophotometer. UV spectra were obtained from Schimadzu UV2450 spectrophotometer with in the range of 200-1000nm. ESR spectrum of Cu(II)-AMTSC complex was obtained from EMX-PLUS-BRUKER X-band RT spectrometer. Magnetic susceptibilities were measured at room temperature on Faraday balance model 7550. Thermo gravimetric analyses of the complexes were carried out on TA model DTG 60 H SHIMADZU in temperature range of 0\degree C-1100\degree C with a ramp of 20\degree C/min. A digital Elico (L1-120) pH meter with a combined glass and calomel electrode was used for equilibrium studies. DNA cleavage experiments were performed with the help of Biotech electrophoresis system supported by Genei power supply over a potential range of 50-500V, visualized and photographed by Biotech Transilluminator system. The antibacterial activity of the title compound and its complexes were tested by Kirby Bauer disc diffusion method.

**Synthesis of 1-acetyl-4-methyl-3-thiosemicarbazide (AMTSC)**

A mixture of 4-methyl-3-thiosemicarbazide (2g, 19mmol) and acetic acid (1.09mL, 19mmol) in acetic anhydride (1.78mL, 19mmol) is refluxed for 30min on water bath (scheme 1) and filtered. On slow evaporation white crystalline solid of AMTSC was obtained, which was recrystallized from ethanol. The progress of the reaction was monitored by TLC. (M.P: 160-162\degree C). It is soluble in methanol and DMF.
Synthesis of metal complexes:

Aqueous metal salt solution (1.7mmol) was added to hot methanolic solution of AMTSC (0.5g, 3.3mmol), [MCl$_2$ M = Cu(II) and Zn(II)] in 1:2 (M:L) molar ratio and was refluxed for 5-10hrs. The pH of the solution was adjusted by the addition of few drops of methanolic ammonium hydroxide. Solution Dark green coloured Cu(II)-AMTSC complex and dirty white coloured Zn(II)-AMTSC complex were separated. The complexes were filtered in hot condition, washed with hot methanol and double distilled water to remove unreacted ligand and metal salts respectively, then washed with petroleum ether and finally dried in vacuum.

RESULTS AND DISCUSSION

Characterization

Liquid chromatogram and mass spectrum

Liquid chromatogram of AMTSC showed a single peak with retention time of 0.816min indicating its purity.

Mass spectrum (Fig.1) of AMTSC revealed the molecular ion peak (APCI-POS1) at m/z 148 (cal 147.2) [M+1]$^+$. Other fragmentation peaks at m/z 170 [M+Na]$^+$, m/z131 [C$_3$H$_5$N$_3$OS]$^+$ are also observed.

IR spectrum: IR spectrum of AMTSC (Fig.2) showed characteristic bands at 3307cm$^{-1}$ ($\nu$NH$_3$), 3146cm$^{-1}$ ($\nu$N-H amide), 3087cm$^{-1}$ ($\nu$N-H thioamide), 2974cm$^{-1}$ ($\nu$CH$_3$), 2941cm$^{-1}$ ($\nu$CH$_3$), 1713cm$^{-1}$ ($\nu$C=O). Other peaks have been assigned to 1286cm$^{-1}$ ($\nu$C=S), 1040cm$^{-1}$ ($\nu$C-C), 973cm$^{-1}$ ($\nu$N-N).
$^{1}$H-NMR: $^{1}$H-NMR spectrum of AMTSC (Fig.3) showed peaks at $\delta$13.41ppm (s,1H,SH), $\delta$10.66ppm (s,1H,NH), $\delta$10.64ppm (s,1H,NH), $\delta$3.66ppm (s,1H,OH), $\delta$2.47ppm (s,3H,NHCH$_3$), $\delta$2.09ppm (s,1H,NH), $\delta$1.19ppm (s,3H,CH$_3$).

The integration in the spectrum indicates the number of protons as nine. However two protons were found to be present in two forms. More number of peaks observed as against expected from number of hydrogen atoms present can be interpreted by keto and enol forms of the compound (Fig.4). Chemical shifts of NH and NH$_2$ protons have been supported by their ready exchange with D$_2$O.

**Figure 4: Keto-enol tautomersism of AMTSC**

**13C-NMR:** Chemical shift values in $^{13}$C-NMR spectrum of the AMTSC (Fig. 5) corresponds to $\delta24.15$ppm (CH$_3$), $\delta33.2$ppm (N-CH$_3$), $\delta169$-$\delta175$ppm (C=O), $\delta181.1$-$\delta182.4$ppm (C=S), each signal of C=O and C=S splits into two, supporting keto-enol tautomerism.

**UV-Visible:** The UV-visible spectrum of AMTSC recorded in DMSO showed (Fig.6) transitions at 273nm (36,563 cm$^{-1}$) corresponds to n→ $\pi^*$ (C=O) and 207nm (48,309 cm$^{-1}$) corresponds to n→ $\pi^*(C=S)$. 

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**Fig.5: $^{13}$CNMR spectrum of AMTSC**

**Fig.6. UV-Visible spectrum of AMTSC**
Computational studies

The molecular modeling program HYPERCHEM 7.5\(^{19,24}\) was used for theoretical calculations. To understand the stability and reactivity of AMTSC semi-empirical (AM1) studies have been performed. Thione-thiol and keto-enol forms of AMTSC were built, and geometry optimized with molecular mechanics (AMBER). Single point energy, heat of formation and dipole moments of the optimized geometries were calculated using quantum mechanical Semi empirical AM1 calculations (Table 1).

The lower energies and lower heat of formation (less positive) values indicate that thione and thiol-1 forms (Fig. 4 Form I and III) of the AMTSC are stable. Close proximity in energies of keto and enol forms (Fig. 4 Form I and II) indicate the possibility of co-existence of both the forms. Calculated dipole moment values indicates polar nature of all the forms. Among all forms, enol form is more polar in nature (Fig.4 Form II).

Table 1. Molecular Properties of AMTSC

<table>
<thead>
<tr>
<th>Molecular properties-AMTSC</th>
<th>Thione form</th>
<th>Enol form</th>
<th>Thiol form 1</th>
<th>Thiol form 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single point energy (Kcal/mole)</td>
<td>-1596.49</td>
<td>-1583.42</td>
<td>-1589.35</td>
<td>-1578.40</td>
</tr>
<tr>
<td>Heat of formation (Kcal/mole)</td>
<td>20.49</td>
<td>34.01</td>
<td>28.08</td>
<td>39.03</td>
</tr>
<tr>
<td>Dipole moment (Debye)</td>
<td>2.72</td>
<td>6.21</td>
<td>3.42</td>
<td>3.95</td>
</tr>
</tbody>
</table>

Table 2. Comparison of HOMO–LUMO energy, hardness, ionization energy and electron affinity of AMTSC

<table>
<thead>
<tr>
<th>Energies-AMTSC</th>
<th>Thione form</th>
<th>Enol form</th>
<th>Thiol form 1</th>
<th>Thiol form 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E_{HOMO}) (eV)</td>
<td>-8.768</td>
<td>-8.646</td>
<td>-9.862</td>
<td>-9.486</td>
</tr>
<tr>
<td>(E_{LUMO}) (eV)</td>
<td>0.120</td>
<td>0.542</td>
<td>0.696</td>
<td>0.528</td>
</tr>
<tr>
<td>(E_{HOMO} - E_{LUMO}) (eV)</td>
<td>8.888</td>
<td>9.188</td>
<td>10.558</td>
<td>10.016</td>
</tr>
<tr>
<td>Hardness = 1/2((E_{HOMO}-E_{LUMO}))</td>
<td>4.444</td>
<td>4.594</td>
<td>5.278</td>
<td>5.008</td>
</tr>
<tr>
<td>IE = - (E_{HOMO})</td>
<td>8.768</td>
<td>8.646</td>
<td>9.862</td>
<td>9.486</td>
</tr>
<tr>
<td>EA = - (E_{LUMO})</td>
<td>-0.120</td>
<td>-0.542</td>
<td>-0.696</td>
<td>-0.528</td>
</tr>
</tbody>
</table>

FMO Analysis: Analysis of HOMO and LUMO of the chemical compounds play an important role in understanding its chemical reactivity. Ionization energy (IE), electron affinity (EA) and hardness can be expressed as \(-E_{HOMO}, -E_{LUMO}\) and 1/2\((E_{HOMO}-E_{LUMO})\) respectively (Table 2).

A system with large gap between HOMO and LUMO will be less reactive and is harder in nature. In the present study, this gap is between 8.88 to 10.55eV (1.67eV) indicating stability and hardness of the molecule in all the four forms which is also supported by their ionization energy. The geometry optimized structures of thione-thiol and enol forms, their electrostatic potential (ESP) mapping and the generated molecular orbital energy diagrams - HOMO, LUMO\(^{25,26}\) are presented in Fig.7. Electrostatic potential mapping gives information about the reactivity of the molecule with nucleophilic/electrophilic reagents. From the electrostatic potential mapping violet region around oxygen and nitrogen atoms with amide linkages indicate negative ESP, hence more susceptible to electrophilic attack by a suitable molecule.
QSAR Studies: QSAR studies help to recognize and compute the physicochemical properties of a drug and its effect on biological activity. From log p values of the ligand (Table: 3), it can be understood that ligand possess\(^2\) good penetrating capability into cell membrane and in turn has considerable biological activity only in its enol form.
Table 3: QSAR Properties

<table>
<thead>
<tr>
<th>Properties (Abinitio) AMTSC</th>
<th>Thione form</th>
<th>Enol form</th>
<th>Thiol form-1</th>
<th>Thiol form-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial charge</td>
<td>0.00e</td>
<td>0.00e</td>
<td>0.00e</td>
<td>0.00e</td>
</tr>
<tr>
<td>Surface area (approx) (Å²)</td>
<td>339.10</td>
<td>327.40</td>
<td>338.17</td>
<td>326.51</td>
</tr>
<tr>
<td>Surface area (grid) (Å²)</td>
<td>327.68</td>
<td>332.94</td>
<td>332.53</td>
<td>330.33</td>
</tr>
<tr>
<td>Volume (Å³)</td>
<td>483.00</td>
<td>484.03</td>
<td>487.07</td>
<td>481.80</td>
</tr>
<tr>
<td>Hydration energy (K.cal/mol)</td>
<td>-11.02</td>
<td>-15.82</td>
<td>-8.87</td>
<td>-8.73</td>
</tr>
<tr>
<td>Log P</td>
<td>-0.12</td>
<td>0.91</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Mass (a.m.u)</td>
<td>147.19</td>
<td>147.19</td>
<td>147.19</td>
<td>147.19</td>
</tr>
</tbody>
</table>

In this compound oxygen and nitrogen atoms with amide linkages are more susceptible to electrophilic attack by a suitable molecule.

Characterization of metal complexes of AMTSC

Cu(II) and Zn(II) complexes of AMTSC were quite stable to air and moisture, amorphous and were soluble in DMF and DMSO decomposed above 300ºC. Molar conductivities recorded in DMSO were in the range of 7-9 ohm⁻¹ cm⁻¹ mol⁻¹ suggesting the non electrolytic nature of the complexes. Volhard’s test revealed the absence of chloride ion.

Mass spectra: Mass spectrum of Cu(II)-AMTSC (Fig.8) recorded at APCI positive mode showed molecular ion peak at m/z 428 indicating [ML₂₄H₂O]⁺, m/z 392 [ML₂₂H₂O]⁺, at m/z 374 [ML₂ H₂O]⁺, m/z 356 [ML₂]⁺, m/z 212 [ML⁺1]⁺ were observed. Peak at m/z 63 corresponds to copper ion.

![Fig.8. Mass spectrum of Cu(II)-AMTSC](image)

APCI positive mode mass spectrum of Zn(II)-AMTSC complex (Fig.9) showed molecular ion peak at m/z 265 [MLH₂O]z⁺, m/z 247 [ML-H₂O]⁺, m/z 214 [ML⁺1]⁺ were observed.

![Fig.9. Mass spectrum of Zn(II)-AMTSC](image)
Thermogravimetric analysis

Thermogram of Cu(II)-AMTSC (Fig. 10) indicated that the complex decomposed in three steps. Weight loss (9.2%) between 100ºC-170ºC indicates the loss of two moles of lattice water molecules. Weight loss between 210ºC-320ºC is (9.2%) indicating loss of two coordinated water molecules. Weight loss between 320ºC-1023ºC is 47%, in various steps due to decomposition of the complex. Percentage of residue left at 1023ºC is 34.6% indicating partial decomposition of the complex.

Zn(II)-AMTSC complex thermogram (Fig. 11) showed decomposition of the complex in four steps. Weight loss between 110ºC-180ºC is 6.79% indicating loss of one mole of lattice water molecule. Weight loss between 200ºC-320ºC is 13.58% indicating loss of two moles of coordinated water molecules. Weight loss between the temp 300ºC-501ºC of 24.69%, and 501ºC-1100ºC of 20%, indicates decomposition of the complex. Percentage of residue left at 1100ºC is 36% indicating partial decomposition of the complex.

IR spectra

In the IR spectrum of AMTSC the $\nu_{\text{NH}} (N4)$ peak observed at 3307 cm$^{-1}$ is shifted to higher frequency region 3363 cm$^{-1}$ in Cu(II)-AMTSC complex (Fig. 12) indicating participation of ‘N’(4) in the bonding without deprotonation. The $\nu_{\text{NH}} (N1)$ peak observed in the IR spectrum of AMTSC at 3146 cm$^{-1}$ was absent in the complex indicating participation of ‘N’(1) in the bonding by the dissociation of one proton. The $\nu_{\text{NH}} (N2)$ peak observed in the IR spectrum of AMTSC at 3087 cm$^{-1}$ has also shifted to higher frequency region at 3120 cm$^{-1}$ in the complex. The $\nu_{\text{C=O}}$ peak has been shifted to higher frequency region in the complex indicating the participation of ‘N’(1) in the bonding. Therefore ‘N’(1) and ‘N’(4) act as potential donor sites forms a five membered chelate with Cu(II) ion (Fig. 4 Form-I).

The $\nu_{\text{NH}} (N4)$ peak observed at 3307 cm$^{-1}$ in the IR spectrum of AMTSC was absent in Zn(II)-AMTSC complex (Fig. 13) may be because of thione-thiol tautomerism. The $\nu_{\text{C=S}}$ peak observed at 1286 cm$^{-1}$ in the IR spectrum of AMTSC was absent in the complex and an extra peak observed at 758 cm$^{-1}$ ($\nu_{\text{C=S}}$) indicating participation of ‘S’ with the dissociation of ‘-SH’ proton in thiol form (Fig. 4 Form IV). The $\nu_{\text{NH}} (N1)$ peak observed in the IR spectrum of AMTSC at 3146 cm$^{-1}$ was absent in the complex and an extra peak corresponds to $\nu_{\text{C=S}}$ has been observed.
at 1552 cm$^{-1}$ in the complex indicating the participation of ‘N’(1) in the bonding. The existence of $\nu_{NH(N2)}$ peak in the IR spectrum of Zn(II)-AMTSC complex and shifted to higher frequency (3100 cm$^{-1}$) indicates participation of ‘N’(2) in the bonding without deprotonation. The $\nu_{C=O}$ peak observed at 1713 cm$^{-1}$ in the IR spectrum of AMTSC was absent in the complex and an extra peak corresponds to $\nu_{C=O}$ has been observed at 1132 cm$^{-1}$ indicating deprotonation of amide proton from oxygen in the enol form and participation of ‘oxygen’ in the bonding. The binding of all the sites to a single metal ion results strain in the complex. Therefore two sites ($\nu_{NH(N1)}$ and ‘S’ of CS sites) coordinate with one metal ion another two sites (‘O’ of CO and $\nu_{NH(N2)}$ sites) coordinate with another metal ion resulting in a polynuclear complex. From Far-IR spectra, there is a clear evidence of presence of extra peaks in the spectra of complexes $\nu_{MN}$(435-485 cm$^{-1}$), $\nu_{MO}$(400-430 cm$^{-1}$), $\nu_{MS}$(355-395 cm$^{-1}$) and $\nu_{M-OH2}$(400-440 cm$^{-1}$).

UV-Visible spectrum

The transitions observed in Cu(II)-AMTSC (Fig.14) are ($^2E_g \rightarrow ^2T_{2g}$) at 12,070 cm$^{-1}$ and 48,076 cm$^{-1}$ due to charge transfer transition along with ligand chromophore at 36,461 cm$^{-1}$ indicating distorted octahedral geometry around d$^9$ system of Cu(II).

ESR spectrum and Magnetic susceptibility measurements

Cu(II)-AMTSC (Cu(II): I=3/2) ESR spectrum of the complex (Fig.15) showed three g
values. The $g_x$, $g_y$ and $g_z$ values 2.1750, 2.1091 and 2.0776 respectively indicating distorted octahedral geometry. The magnetic moment value of Cu(II)-AMTSC is 1.73 BM indicating presence of one unpaired electron in the complex.

**Equilibrium studies**

To understand the chelation properties of AMTSC in solution an attempt has been made to study its potential donor sites that bind with metal ions. Irving-Rossotti pH titration technique was employed for the determination of dissociation constant of AMTSC and its stability constants with Zn(II) ions in 70% v/v DMF-water medium at 303K and 0.1M KNO$_3$ ionic strength. From the titration data obtained (Table 4), dissociation constant have been calculated by plotting linear graphs of Log ($1/nA$)/($nA$) Vs pH (Fig.16). The results indicated the presence of one dissociable proton corresponding to amide (-NH) proton via enol formation. (pKa = 9.94). Values of $\bar{n}$ ranges from 0.2 to 0.9 ($\log k_1$ = 5.94) (Table. 5), indicating formation of 1:1 complex in solution. The stability constant of the binary complex was calculated from linear plot (Fig.17) of log $([(1-\bar{n})/\bar{n}]$ Vs pL. After the formation of 1:1 Zn(II)-AMTSC complex in solution, further calculations revealed that the results increased in an abnormal manner hence only 1:1 formation constant is reported. Abnormal increase in pH values supports the polymeric nature of Zn(II)-AMTSC complex, concluded from solid studies. These values are further refined by using MINIQUAD program.

**Table 4. Data for obtaining Dissociation constants of AMTSC**

<table>
<thead>
<tr>
<th>Ligand/Medium</th>
<th>pH</th>
<th>$\bar{n}$</th>
<th>$\log(1-\bar{n})/\bar{n}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMTSC/DMF</td>
<td>9.4</td>
<td>0.7604</td>
<td>-0.5015</td>
</tr>
<tr>
<td></td>
<td>9.5</td>
<td>0.7206</td>
<td>-0.4114</td>
</tr>
<tr>
<td></td>
<td>9.6</td>
<td>0.6807</td>
<td>-0.3288</td>
</tr>
<tr>
<td></td>
<td>9.7</td>
<td>0.6009</td>
<td>-0.1777</td>
</tr>
<tr>
<td></td>
<td>9.8</td>
<td>0.5610</td>
<td>-0.1065</td>
</tr>
<tr>
<td></td>
<td>9.9</td>
<td>0.4811</td>
<td>0.0328</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.4614</td>
<td>0.0671</td>
</tr>
<tr>
<td></td>
<td>10.1</td>
<td>0.4415</td>
<td>0.1020</td>
</tr>
<tr>
<td></td>
<td>10.2</td>
<td>0.3816</td>
<td>0.2096</td>
</tr>
</tbody>
</table>
Table 5. Data for obtaining, Formation curves of Zn(II)-AMTSC

<table>
<thead>
<tr>
<th>( n )</th>
<th>( \log(1-n)/n )</th>
<th>pL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>0.6020</td>
<td>6.38</td>
</tr>
<tr>
<td>0.3</td>
<td>0.3679</td>
<td>6.22</td>
</tr>
<tr>
<td>0.4</td>
<td>0.1760</td>
<td>6.06</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>5.95</td>
</tr>
<tr>
<td>0.6</td>
<td>-0.1760</td>
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</tr>
<tr>
<td>0.7</td>
<td>-0.3679</td>
<td>5.68</td>
</tr>
<tr>
<td>0.8</td>
<td>-0.6020</td>
<td>5.49</td>
</tr>
</tbody>
</table>

Fig. 16. Linear Plot of \( \log \left( \frac{1-n}{n} \right) \) vs pH

Fig. 17. Linear Plot of Zn(II)-AMTSC

From the above discussions based on all the analytical, spectral techniques employed and equilibrium studies the following tentative structures (Fig. 18) for the metal complexes have been proposed.

Fig. 18. Tentative structures of Cu(II)-AMTSC and Zn(II)-AMTSC
DNA cleavage studies

Super coiled (SC) plasmid DNA, commonly seen in bacteria cells, will be cyclic super coiled double strand made up of several thousand base pairs. This has been important substrate for hydrolytic cleavage. Metal ions in the complexes serve as Lewis acids to activate the phospho-diester links for nucleophilic attack and metal coordinated water species acts as a nucleophile. When DNA is subjected to electrophoresis, the intact SC form migrates faster. When scission occurs due to action of complex, SC form will relax to nicked (NC) form that migrates slowly. Cleavage of both types of strands leads to linear form which migrates between SC and NC forms, because shorter molecules migrate more easily through the pores of the gel. In the present investigation it is observed (Fig 19) that both the complexes of AMTSC promote hydrolytic cleavage of plasmid pBR322 to certain extent due to scission in SC forms of DNA to NC forms.

![Agarose gel electrophoresis pattern for the cleavage of supercoiled pBR 322 DNA by complexes. Lane 1, DNA control, Lane 2-4 DNA+ Cu(II) (20,40,60 µM resp.), Lane 5-7 DNA+ Zn(II) (20,40,60 µM resp.) of AMTSC.](image)

Antibacterial studies

The Cu(II)-AMTSC and Zn(II)-AMTSC were found to inhibits the growth of gram positive and gram negative bacteria (Table 6). Cu(II)-AMTSC showed more activity on gram positive bacteria compared to AMTSC. This can be attributed to the chelating capacity of the ligand with metal ion. Metal atom partially shares its positive charge with the donor atoms of the ligand. This leads to delocalization of π electron cloud over the chelating ring. Due to this, the lipophilic character of the metal gets enhanced and favours its permeability into bacterial cell membranes and inhibits the growth of the bacteria.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Gram positive</th>
<th>Gram negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Staphylococcus Aureus</td>
<td>Bacillus subtilis</td>
</tr>
<tr>
<td>AMTSC</td>
<td>6 mm</td>
<td>7 mm</td>
</tr>
<tr>
<td>Cu(II)-AMTSC</td>
<td>9 mm</td>
<td>8 mm</td>
</tr>
<tr>
<td>Zn(II)-AMTSC</td>
<td>7 mm</td>
<td>NIL</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Spectral and analytical studies indicate that AMTSC forms distorted octahedral complexes in 1:2 (M: L) composition with copper(II) and in 1:1(M:L) composition with
Zinc(II) ions. Equilibrium studies reveal that AMTSC acts as monobasic ligand and forms stable 1:1(M-L) complex with Zinc(II) ions in solution. Energy parameters from computational calculations showed possible coexistence of thione-thiol and keto-enol forms. From computational studies it can be concluded that AMTSC is stable, polar and harder molecule. It possess good penetrating capacity into cell membrane and has considerable biological activity. DNA cleavage studies reveal that both the complexes can cleave SC form of plasmid DNA.

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REFERENCES