Metal Complexes and Organotin(IV) Compounds of Cefixime and their Biological Study

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Abstract

Synthetic chemistry is playing revolutionary role in human beings by synthesizing novel compounds by different techniques. The present study was designed to prepare bioactive compounds and evaluate their antioxidant and enzyme inhibition studies. Metal complexes of cefixime were prepared by reacting metal salts of different transition metals with sodium salt of cefixime. Their characterization was done with melting point and FTIR studies which confirmed the synthesis of metal complexes. It was concluded from results that synthesized compounds showed very weak response against AChE and some extent to BChE. In contrast to esterase family protease was inhibited significantly and T4 exhibited more than 90% inhibition (93.2 ± 0.1%). Antioxidant study was conducted using well known DPPH free radical assay while enzyme inhibition potential was evaluated according to standard protocols. Maximum antiradical activity was exhibited by T6 (80.84 ± 1.6%) with IC50 45 ± 2 μg/ml while T5 remained almost inactive. Antibacterial studies have been carried out against Escherichia coli and Bacillus subtilis.

Keywords: Metal complexes; Cefixime; DPPH, Proteases; AChE; BChE

Introduction

Cefixime has broad spectrum of activity, used mostly orally, high stability against β-lactamases and is susceptible to many organisms. Chemically, it is (6R,7R)-7-[2-(2-Amino-4-thia-zolyl)glyoxylamido]-8-oxo-3-vinyl-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid, 72-(Z)-(O-carboxymethyl) oxime trihydrate.

Cefixime

Cefixime like other cephalosporins inhibits the synthesis of bacterial cell wall which is the important cellular structure that strengthens the bacterial body and keep them safe from environmental hazards. It is efficiently used against number of bacteria including Streptococcus pneumoniae, Staphylococcus aureus, Hemophilus influenzae, Moraxella catarrhalis, Streptococcus pyogenes, Escherichia coli, Klebsiella, Proteus mirabilis, Salmonella, Neisseria gonorrhoeae and Shigella. It is used to treat otitis media, pharyngitis, bronchitis and urinary tract infections [1-4].

In biological systems, chain reactions can lead to severe damage or death of cells. Antioxidants act by inhibiting these chain reactions by trapping free radicals. Highly unstable free radicals and reactive oxygen species are present in living body from variety of sources. These free radicals may oxidize vital molecules of body like DNA, protein, lipids and nucleic acid leading body to degenerative diseases.

Antioxidants seem to protect body from chronic diseases including heart disease and cancer. Free radical scavenging activity was first reported by Miller et al [5].

Enzyme inhibitor is a organic chemical or inorganic metal or biosynthetic compound due to their covalent or non-covalent interactions with enzyme active site. They can reduce or completely inhibit the enzyme catalytic activity either reversibly or permanently (irreversibly) by modifying one amino acid, or several side chain(s) required in enzyme catalytic activity. In drug discovery, several drug analogues are chosen and/or designed to inhibit specific enzymes. Antibacterial substance is one of feed additives to be used in order to promote beneficial use of nutrient components in feed. Many authors are engaged now a days in preparation of metal complexes of some antibiotic in order to check potential of derivatives drugs [6-9]. Our research group is also working on such line to evaluate the comparability of the resistance in the bacterial and derivation. The aim of this study was to check the biological potential of metal complexes of Cefixime in terms of antibacterial, antioxidant and enzyme inhibition potential.

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Experimental

Chemicals and instruments

DPPH (Diphenyl picryl hydrazyl), 5,5’-dithiobis [2-nitrobenzoic acid] (DTNB), protease and DMSO were purchased from Sigma-Aldrich (USA) while AChE (Acetylcholine esterase) and BChE (Butyrylcholine esterase) were obtained from Biochemistry Laboratory Mayo Hospital Lahore. All other chemicals and solvents used were of analytical grade from Merck. UV/Vis. Spectrophotometer (UV-4000, Shimadzu), atomic absorption spectrophotometer (Shimadzu, AA-6300) and FTIR (Shimadzu, IR-Prestige-21) were used during studies.

Synthetic scheme

The targeted compounds were prepared according to literature method [10,11]. Complexes of transition metal were prepared by drop-wise addition of aqueous solution of sodium salt of cefixime to an aqueous solution of transition metal salt (copper (II) chloride, cadmium (II) nitrate and iron (II) sulphate) in a 250 ml round bottom flask equipped with a water condenser and magnetic stirring bar. The reflux was carried out for about 3 hr till complete precipitation occurred. After the formation of precipitates, the reaction mixture was cooled, filtered and the residue was dried completely. Complexes of organotin halides with sodium salt of ligand acid were prepared by the addition of appropriate triorganotin chloride in methanol to the reaction mixture drop-wise with constant stirring. The reaction mixture was then refluxed for 4-6 hr, cooled and filtered. The filtrate was concentrated on rotary evaporator and then kept for crystallization. Recrystallization was carried out from a suitable solvent.

\[
\begin{align*}
MX_2 + NaL & \rightarrow ML + 2NaX \\
Bu_2SnX_2 + NaL & \rightarrow Bu_2SnL + 2NaX \\
R_3SnX + NaL & \rightarrow 2R_3SnL + 2NaX \\
T_2: Mn(CO)_3, T_4: MnCu_2, T_5: MnFe_2, T_3: R=CH_2, T_6: R=\text{Bu}
\end{align*}
\]

Characterization

Characterization of synthesized compounds were done with the help of UV/Vis and FTIR techniques while metal to ligand ration was calculated with atomic absorption spectrophotometer. In the spectrum mode of UV/Vis spectrophotometer, \( \lambda_{\text{max}} \) was determined. Blank solution was used to correct base line in the range of 190 nm-800 nm. Then sample cell was filled with very dilute sample solution and scan was obtained for UV/Vis spectrum (plot of wavelength vs absorbance). \( \lambda_{\text{max}} \) was detected from the peak maximum of the spectrum [12].

Antibacterial studies

The representative samples were individually tested against two bacterial strains including Escherichia coli and Bacillus subtilis using agar well diffusion method. Bacterial strains were available and maintained in the Microbiology laboratory, Department of Zoology, Mayo Hospital Lahore. All other chemicals and solvents used were of analytical grade from Merck. UV/Vis. Spectrophotometer (UV-4000, Shimadzu), atomic absorption spectrophotometer (Shimadzu, AA-6300) and FTIR (Shimadzu, IR-Prestige-21) were used during studies.

Antioxidant activity

The radical scavenging ability was measured by using the method of Shahwar et al. [14]. 100 µl of the synthesized compound (5 mg/ml in DMSO) was added into 2 ml methanolic solution of DPPH. The reaction mixture was incubated at room temperature for 30 minutes. The decrease in absorbance at 517 nm was noted after 30 min.

\[
\text{% Inhibition} = \frac{A - B}{A} \times 100
\]

Acetylcholine/Butyrylcholine esterase assay

Acetyl/butyrylcholine ester inhibitory activity was measured by spectrophotometric method of Abbasi et al. [16]. Acetyl/butyrylthiocholine iodide was used as substrate in the assay. The reaction mixture contained 1500 µl of (100 mM) tris buffer (pH 7.8), 100 µl of DTNB, 100 µl (5 mg/ml) of test compound solution and 100 µl of enzyme, which were mixed and incubated for 15 min (37 °C). The reaction was initiated by the addition of 100 µl acetyl/butyrylthiocholine iodide. The hydrolysis of acetyl/butyrylthiocholine iodide was monitored at 412 nm after 30 min. All the reactions were performed in triplicate. The percentage inhibition was calculated as follows:

\[
\text{% Inhibition} = \frac{E \times S}{E} \times 100
\]

Where E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound.

Results and Discussion

Characterization

The infrared studies of the complexes show that this drug has donor atoms that can bind to metal ions forming multinuclear chelates. It bonds to metal ion through the carboxylate ion, the nitrogen of the β-lactam thiazolidine ring and oxygen atom of lactam C=O group rather than amid carbonyl group [17].

In the spectrum mode of UV/Vis spectrophotometer, \( \lambda_{\text{max}} \) was determined. The value of \( \lambda_{\text{max}} \) for ligands understudy and synthesized complexes are presented in Table 1. The value of \( \lambda_{\text{max}} \) for all the compounds was found between 291-298 nm that indicated the presence of various chromophoric groups (C=O, C=C, C=N) in nutrient agar medium [13].
may arise due to the co-ordination of the ligand with metal ions to form complexes.

The infrared spectra of the ligands and prepared compounds were recorded as KBr pellets in the range 4000–400 cm⁻¹. FTIR absorptions of these complexes were assigned in comparison with that of free acids and their sodium salts. IR absorptions of those wave numbers are discussed mainly here which are directly involved in complex formation. The absorption bands were assigned by comparison with earlier reports [18] and important frequencies such as ν(C=O) lactam, ν(C=O)-NH, ν(COO) asymmetric ν(COO) symmetric, and ν(Sn-O) are listed in Table 2. The broad band at 2730-2980 cm⁻¹ due to ν(OH) present in the spectrum of the ligand is absent in the spectra of corresponding synthesized complexes thus showing complex formation.

After complexation, the C = O of carboxylic acid shifts between 1660-1539 cm⁻¹ as compared to free acid at 1670 cm⁻¹. Similarly, coordination of β-lactam of carbonyl group takes place as compared to ligand and sodium salt hence C = O double bond character decreases and this vibration shifts to lower region between 1770-1730 cm⁻¹.

Values of group ν(COO) symmetric vibrations lowered due to presence of delocalization of π-electrons in the adjacent C=C and C=O bonds, this delocalization increases single bond character in them hence lowers the frequencies of C=O and C=C groups.

The lactam ν(C=O) band appears in range of 1774-1770 cm⁻¹ in free acids and their sodium salts while in spectra of complexes this bond is shifted to lower absorptions indicating that binding occurs through the oxygen atom of lactam C=O group. The asymmetric stretching absorptions of carboxylate group and lactam group are decreased depending on the nature of metal ion. The shifting of absorptions due to amide carbonyl bond ν(C=O)-NH in complexes is not remarkable in comparison to free acid and its salt thus indicating that ligand coordination with metal ions takes place through oxygen atom of lactam C=O group rather than amide carbonyl group [19]. In case of respective organotin(IV) complexes, absorptions due to Sn-O band are recorded in the range of 500-400 cm⁻¹.

The IR studies of cefixime ligand and its respective complexes suggested that this antibiotic has number of donar atoms that can bind to the metal ions forming multinuclear chelates.

In order to ascertain about the geometry of these amorphous compounds, metal to ligand ratio was determined using atomic absorption spectrophotometry and results are shown as in the Table 1. These results show that in case of transition metal complex as well as diorganotin(IV) complex, metal to ligand ratio is 2:1, thus showing distorted octahedral geometry. However, in case of triorganotin complexes metal to ligand ratio is 2:1 which shows that each carboxylate group consumes one organotin moiety resulting in distorted trigonal bipyramidal geometry. It is, however, pertinent to mention that in absence of a clear cut X-ray report geometry of these complexes can not be ascertained and literature is also scarce about such systems.

### Table 1: Physical properties, Amax values and metal to ligand ration of the ligand/complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Code</th>
<th>color</th>
<th>State</th>
<th>Solubility</th>
<th>Yield (%)</th>
<th>m.p (°C)</th>
<th>Amax (nm)</th>
<th>M:L ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>T1</td>
<td>Yellowish</td>
<td>Powder</td>
<td>C₇H₇OH</td>
<td>-</td>
<td>220</td>
<td>291</td>
<td>-</td>
</tr>
<tr>
<td>CdL</td>
<td>T2</td>
<td>Green</td>
<td>Powder</td>
<td>C₇H₇OH</td>
<td>83</td>
<td>240</td>
<td>297</td>
<td>1:1</td>
</tr>
<tr>
<td>(Me)₂SnL</td>
<td>T3</td>
<td>Brown</td>
<td>Powder</td>
<td>C₇H₇OH</td>
<td>75</td>
<td>167</td>
<td>195</td>
<td>2:1</td>
</tr>
<tr>
<td>CuL</td>
<td>T4</td>
<td>Brown</td>
<td>Powder</td>
<td>C₇H₇OH</td>
<td>74</td>
<td>363</td>
<td>196</td>
<td>1:1</td>
</tr>
<tr>
<td>FeL</td>
<td>T5</td>
<td>Brown</td>
<td>Powder</td>
<td>C₇H₇OH</td>
<td>62</td>
<td>340</td>
<td>197</td>
<td>1:1</td>
</tr>
<tr>
<td>(Bu)₂SnL</td>
<td>T6</td>
<td>Brown</td>
<td>Powder</td>
<td>C₇H₇OH</td>
<td>70</td>
<td>158</td>
<td>197</td>
<td>2:1</td>
</tr>
<tr>
<td>(Bu)₃SnL</td>
<td>T7</td>
<td>Brown</td>
<td>Powder</td>
<td>C₇H₇OH</td>
<td>74</td>
<td>167</td>
<td>196</td>
<td>1:1</td>
</tr>
</tbody>
</table>

*Proposed structures of the complexes*

**Microbiological screening**

Size of inhibition diameter was measured to determine the susceptibility of strains of bacterium towards cefixime and their representative metal complexes. During biological testing the complexes remain intact as assessed by color. Bactericidal diameter measured for ligands and complexes shows that they have good activity as bactericide as shown in Table 3.

The results have shown that the metal complexes have two types of behaviour compared with ligands against the same bacteria and under the identical experimental conditions. For example, some of metal complexes show higher bactericidal activity than that of corresponding ligand and some complexes have less activity than ligand. These differences may arise due to  steric, electronic, pharmacokinetic factors along with mechanistic pathways.

**Protease, AChE and BChE inhibition activity**

All synthesized compounds were screened against available enzymes (Protease, AChE and BChE). Interesting results were obtained during this study. About all complexes showed significant activity. Among them T4 (CuL) exhibited maximum activity (93.2 ± 0.1%) with IC₅₀ 78 ± 1 μg/ml for protease as shown in Table 4 and Figure 1. During this evaluation it was found that ligand remained almost inactive but its complexes has greater activity than ligands, which showed that metal ion has changed the configuration of cefixime antibiotic for binding to active sites of enzyme.

These complexes along their ligand were also evaluated in terms of AChE and BChE inhibition studies. It was observed that very low response was exhibited by all complexes against AChE and same behavior was observed for BChE except T2 (CdL). These results indicated that these agents have very low potential to inhibit the active site of enzyme. Enzyme inhibition play vital role in curing various diseases in human beings. Extensive research on this topic is being...
Antioxidant activity of synthesized compounds was checked by using well reported stable free radical DPPH. All compounds showed remarkable activity except T3 [(Me)SnL] and T5 (FeL) which almost remained inactive. It was also concluded that there is small difference between activity of ligand and their metal complexes. Maximum activity (80.84 ± 1.6%) was exhibited by T6 [(Bu)SnL] with IC50 value 45 ± 2 μg/ml (Table 5 and Figures 2 and 3). The order of potential of compounds in antiradical scavenging activity is: T6>T7>T4>T2>T1>T3>T5. Our results suggest that these compounds can be used as antioxidant.

### Table 2: Fundamental IR vibrations of the cefixime complexes.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Code</th>
<th>β lactam ν(C=O) cm(^{-1})</th>
<th>ν(HNC=O) cm(^{-1})</th>
<th>ν(COO)(^{\text{asym}}) cm(^{-1})</th>
<th>ν(COO)(^{\text{sym}}) cm(^{-1})</th>
<th>ν(C-O) cm(^{-1})</th>
<th>ν(Sn-O) cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>T1</td>
<td>1774</td>
<td>1587</td>
<td>1660</td>
<td>1410</td>
<td>1030</td>
<td>-</td>
</tr>
<tr>
<td>CdL</td>
<td>T2</td>
<td>1768</td>
<td>1535</td>
<td>1680</td>
<td>1377</td>
<td>1045</td>
<td>-</td>
</tr>
<tr>
<td>(Me)SnL</td>
<td>T3</td>
<td>1720</td>
<td>1532</td>
<td>1620</td>
<td>1314</td>
<td>1010</td>
<td>417</td>
</tr>
<tr>
<td>CuL</td>
<td>T4</td>
<td>1741</td>
<td>1535</td>
<td>1678</td>
<td>1311</td>
<td>1060</td>
<td>-</td>
</tr>
<tr>
<td>FeL</td>
<td>T5</td>
<td>1755</td>
<td>1539</td>
<td>1672</td>
<td>1421</td>
<td>1002</td>
<td>-</td>
</tr>
<tr>
<td>(Bu)SnL</td>
<td>T6</td>
<td>1703</td>
<td>1536</td>
<td>1539</td>
<td>1406</td>
<td>1055</td>
<td>459</td>
</tr>
<tr>
<td>(Bu)2SnL</td>
<td>T7</td>
<td>1766</td>
<td>1535</td>
<td>1589</td>
<td>1319</td>
<td>1053</td>
<td>450</td>
</tr>
</tbody>
</table>

Table 3: Antibacterial activities of the ligand and complexes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Code</th>
<th>Zone of inhibition E.C. (mm)</th>
<th>Zone of inhibition B.S. (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>T1</td>
<td>15-29 (H.A)</td>
<td>02-06 (M.A)</td>
</tr>
<tr>
<td>T2</td>
<td>11-29 (H.A)</td>
<td>09-27 (H.A)</td>
<td>07-25 (M.A)</td>
</tr>
<tr>
<td>T3</td>
<td>10-24 (H.A)</td>
<td>09-21 (M.A)</td>
<td>12-18 (M.A)</td>
</tr>
<tr>
<td>T4</td>
<td>06-25 (H.A)</td>
<td>15-16 (H.A)</td>
<td>11-12 (M.A)</td>
</tr>
<tr>
<td>T5</td>
<td>10-08 (H.A)</td>
<td>05-10 (M.A)</td>
<td>06-12 (M.A)</td>
</tr>
</tbody>
</table>

*E.C. Escherichia coli, B.S. Bacillus subtilis |
*H.A: Highly active, M.A: Moderately active, L.A: Less active

Table 4: Enzyme inhibition activity.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Code</th>
<th>Protease IC50 (μg/ml)</th>
<th>AChE IC50 (μg/ml)</th>
<th>BChE IC50 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>T1</td>
<td>64.4 ± 0.7</td>
<td>9.9 ± 0.1</td>
<td>26.84 ± 0.2</td>
</tr>
<tr>
<td>CdL</td>
<td>T2</td>
<td>62.6 ± 0.4</td>
<td>3.3 ± 0.3</td>
<td>57.40 ± 0.3</td>
</tr>
<tr>
<td>(Me)SnL</td>
<td>T3</td>
<td>60.5 ± 0.3</td>
<td>12.7 ± 0.3</td>
<td>22.78 ± 0.4</td>
</tr>
<tr>
<td>CuL</td>
<td>T4</td>
<td>93.2 ± 0.1</td>
<td>3.1 ± 0.5</td>
<td>13.17 ± 0.6</td>
</tr>
<tr>
<td>FeL</td>
<td>T5</td>
<td>28.1 ± 0.5</td>
<td>5.8 ± 0.2</td>
<td>24.84 ± 0.2</td>
</tr>
<tr>
<td>(Bu)SnL</td>
<td>T6</td>
<td>59.0 ± 0.2</td>
<td>11.4 ± 0.1</td>
<td>17.91 ± 0.3</td>
</tr>
<tr>
<td>(Bu)2SnL</td>
<td>T7</td>
<td>44.9 ± 0.6</td>
<td>30.7 ± 0.4</td>
<td>19.28 ± 0.5</td>
</tr>
</tbody>
</table>

* 100 μl samples (5 mg/ml DMSO), - = Not Calculated

Figure 1: Protease, AChE and BChE inhibition activity.

Figure 2: Graphical representation of DPPH antioxidant activity.
Conclusion

In modern era with the development of coordination chemistry, it is accepted that complexes not only increase drug efficiency but also enhance its effectiveness against particular disease as compared to simple drug. These complexes were found more active as compared to the simple ligand against various microbes. Similarly, these possess more antioxidant potential than that of drug alone. Furthermore, these metal complexes are good protease inhibitors and showed moderate response against AChE as well as BChE. This study suggested that these bioactive metal complexes may be used as free radical scavengers as well as antimicrobial agents.

References


Table 5: DPPH antioxidant activity.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>%age Inhibition</th>
<th>IC50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>56.21 ± 1.1</td>
<td>49 ± 2</td>
</tr>
<tr>
<td>T2</td>
<td>61.05 ± 1.3</td>
<td>80 ± 3</td>
</tr>
<tr>
<td>T3</td>
<td>38.12 ± 1.0</td>
<td>-</td>
</tr>
<tr>
<td>T4</td>
<td>71.10 ± 1.4</td>
<td>46 ± 1</td>
</tr>
<tr>
<td>T5</td>
<td>12.63 ± 1.3</td>
<td>-</td>
</tr>
<tr>
<td>T6</td>
<td>80.84 ± 1.6</td>
<td>45 ± 2</td>
</tr>
<tr>
<td>T7</td>
<td>78.37 ± 1.7</td>
<td>49 ± 3</td>
</tr>
</tbody>
</table>

Figure 3: Graphical representation IC50 values of synthesized compounds.

* 100 µl samples (5 mg/ml DMSO), - = not calculated

DPPH Activity