Synthesis, Characterization and Antimicrobial Activity of Some Mixed Trimethoprim -Sulfamethoxazole Metal Drug Complexes

Aderoju A. Osowole, Sherifah M. Wakil and Olaoluwa K. Alao

Abstract: Mixed drug metal(II) complexes of Trimethoprim (TMP, HL) and Sulfamethoxazole (SMX, HL') were synthesized and characterized by percentage metal, infrared and electronic spectroscopies, room temperature magnetic moments, melting points and conductance measurements. The metal analysis revealed that the complexes analyze as [M(HL)(HL')(X).nH2O,where M = Mn, Co, Ni, Cu, Fe, Zn and X= Cl/ SO4. Infrared spectra data confirmed that coordination was via the SMX’s sulphone oxygen and amino nitrogen atoms; and TMP’s amino and azomethine nitrogen atoms. The room temperature magnetic moment and electronic spectra data indicated that the metal (II) complexes were all magnetically dilute and octahedral. The molar conductance of the metal (II) complexes in DMSO confirmed that all the complexes were covalent. Interestingly, the in-vitro antimicrobial studies on these metal (II) complexes, TMP and SMX against Bacillus spp, Escherichia sp, Proteus mirabilis, Pseudomonas spp, Streptococcus pyogenes, Staphylococcus aureus and Candida albicans showed that all the metal complexes, TMP and SMX had broad-spectrum antimicrobial activities against these microbes, with the exception of the Ni (II) complex, with inhibitory zones range of 6.0-32.0 mm, 9.0- 29.0, 11.0-25.0 mm respectively.

Keywords: Broad-spectrum - Covalent - Magnetically-dilute - Octahedral - Sulfamethoxazole - Trimethoprim

INTRODUCTION

Extensive research on novel and innovative drugs is predominantly a consequence of new emerging strains of resistant micro-organisms [1]. The use of metal complexes in medicine as chemotherapeutic agent, magnetic resonance imaging contrast agent, rheumatic drugs are well established [2 -6]. Trimethoprim is a broad-spectrum antimicrobial agent with anti-parasitic activities [7]. Trimethoprim in combination with Sulfamethoxazole (co-trimoxazole) is one of the most frequently prescribed antibiotics in nephrology and Pneumocystis jirovecii infections [8-10]. Detailed literature search revealed that some work have been done on the metal (II) complexes of Trimethoprim [11-13] or Sulfamethoxazole [14, 15]. Some work has been reported on co-trimoxazole and its metal complexes [16, 17]. Thus, our aim is to investigate the suitability of TMP and SMX in forming polymeric, high spin and low spin complexes. In addition, the potentials of these mixed ligand metal complexes as broad-spectrum antimicrobial agent’s in-vitro will be verified as a continuation of our research in the field of bioinorganic chemistry [18-22].

MATERIALS AND METHODS

Materials and Reagents: Sulfamethoxazole and Trimethoprim were gifts from Chemopharma Laboratories, Lagos, Nigeria while Copper (II) sulphate pentahydrate, Nickel (II) chloride hexahydrate, Cobalt (II) sulphate heptahydrate, Manganese (II) chloride tetrahydrate, Iron (II) sulphate heptahydrate and Zinc (II) sulphate heptahydrate were obtained from Aldrich chemicals and solvents were purified by distillation.

Preparation of [Mn (HL)(HL') Cl2]. H2O: 0.5 g (1.72 mmole) of TMP (HL) and 0.4362 g (1.72 mmole) SMX (HL') were dissolved in 30 mL of methanol. To the resulting homogenous solution, 0.3408 g (1.72 mmole) of the Mn (II) Chloride tetrahydrate was added dry in bits
while stirring and heating at 60°C. The resulting homogenous solution was then refluxed for 6 h, during which the product formed. This was filtered, washed with methanol and dried over silica gel. The same procedure was used for the preparation of Co (II), Ni (II), Cu (II), Fe (II) and Zn (II) complexes from their chloride and sulphate salts respectively.

Physical Measurement: The electronic spectra of the complexes in DMSO were recorded on a Perkin-Elmer A25 spectrophotometer and infrared spectra were recorded as Kbr disc on a Perkin-Elmer FT-IR BX spectrometer. The room temperature magnetic susceptibilities at 303K were measured on Sherwood Susceptibility Balance MSB Mark 1 and diamagnetic corrections were calculated using Pascal’s constant, melting points were determined with Mel-Temp electrothermal machine and molar conductance of 1 x 10⁻¹ M solutions in DMSO was obtained using electrochemical analyzer Consort C933.

Antimicrobial Assay: The antimicrobial activity of the synthesized complexes as well as their metal-free ligands was studied using the agar diffusion technique. The bacteria and fungus used were identified laboratory strains of Bacillus cereus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus and Candida albicans. The surface of the agar in a petri dish was uniformly inoculated with 0.2 mL of 18 hour old test microbial culture. Using a sterile cork borer, 5 mm wells were bored into the agar. Then 0.06 mL of 10 mg/mL concentration of each metal complex in DMSO was introduced into the wells and the plates were allowed to stand on the bench for 30 minutes before incubation at 37°C for 24 hours, after which inhibitory zones (in mm) were taken as a measure of antimicrobial activity. The experiments were conducted in duplicates and Streptomycin was used as the reference drug.

RESULTS AND DISCUSSION

The reaction of the Trimethoprim (HL), Sulfamethoxazole (HL') with the metal(II) chlorides (Mn and Ni) and metal(II) sulphate of Fe, Zn, Co and Cu gave coloured complexes in low to moderate yields (30-50%) according to equations 1-2.

\[
\text{MCl}_2 \cdot \text{aH}_2\text{O} + \text{HL} + \text{HL}' \rightarrow [\text{M (HL) (HL') Cl}_2] \cdot \text{nH}_2\text{O} + \text{bH}_2\text{O}
\]

(1)

\[
\text{MSO}_4 \cdot \text{aH}_2\text{O} + \text{HL} + \text{HL}' \rightarrow [\text{M (HL) (HL') SO}_4] \cdot \text{nH}_2\text{O} + \text{bH}_2\text{O}
\]

(2)

The ligands, Trimethoprim (HL) and Sulfamethoxazole (HL'), melted at 238-240°C and 169°C respectively, whereas their metal complexes all decomposed in the range 160-178°C, confirming coordination (Table 1).

The geometry of the metal complexes were determined by percentage metal, room temperature magnetic moment measurement, infrared and electronic spectroscopies since we were unable to isolate suitable single crystal for x-ray diffraction measurement (Figure 1).
Table 1: Analytical data of the legends and complexes

<table>
<thead>
<tr>
<th>Compound (Empirical formula)</th>
<th>F. mass</th>
<th>Color</th>
<th>M. p (°)</th>
<th>% yield</th>
<th>µeff (B.M)</th>
<th>%M (Exp)</th>
<th>^m</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>290.32</td>
<td>White</td>
<td>238-240</td>
<td>-</td>
<td>D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HL^1</td>
<td>253.28</td>
<td>White</td>
<td>169</td>
<td>-</td>
<td>D</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[Mn(HL)(HL^1)Cl2]3H2O</td>
<td>723.60</td>
<td>Cream</td>
<td>160*</td>
<td>30</td>
<td>1.87</td>
<td>7.59</td>
<td>15.30</td>
</tr>
<tr>
<td>[Co(HL)(HL^1)SO4]H2O</td>
<td>734.57</td>
<td>Pink</td>
<td>178*</td>
<td>30</td>
<td>5.3</td>
<td>8.02</td>
<td>19.90</td>
</tr>
<tr>
<td>[Ni(HL)(HL^1)Cl2]2H2O</td>
<td>709.34</td>
<td>Blue</td>
<td>168*</td>
<td>30</td>
<td>3.38</td>
<td>8.28</td>
<td>19.23</td>
</tr>
<tr>
<td>[Cu(HL)(HL^1)SO4]H2O</td>
<td>721.13</td>
<td>Green</td>
<td>174*</td>
<td>50</td>
<td>2.3</td>
<td>8.81</td>
<td>13.15</td>
</tr>
<tr>
<td>[Zn(HL)(HL^1)SO4]2H2O</td>
<td>704.97</td>
<td>White</td>
<td>166*</td>
<td>30</td>
<td>D</td>
<td>9.27</td>
<td>12.22</td>
</tr>
</tbody>
</table>

HL = Trimethoprim, HL^1 = Sulfamethoxazole, D = diamagnetic, *= decomposition temperature, Molar conductance (^m) = ohm^-1 cm^2 mol^-1, Exp = experimental, µeff = effective magnetic moments, F. mass = Formular mass, M. p = melting point.

Table 2: Relevant infrared and electronic spectra data of the Complexes.

<table>
<thead>
<tr>
<th>Compound (NH)</th>
<th>(S=O)</th>
<th>(C=N)</th>
<th>(M-N)</th>
<th>(M-O)</th>
<th>(M-Cl)</th>
<th>Electronic spectra (kK)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL</td>
<td>3318s</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25.58, 32.79</td>
</tr>
<tr>
<td></td>
<td>3376s</td>
<td>1309s</td>
<td>582s</td>
<td>579s</td>
<td>422s</td>
<td>12.58, 16.86, 33.67</td>
</tr>
<tr>
<td></td>
<td>3298s</td>
<td>1161s</td>
<td>546s</td>
<td>473s</td>
<td>-</td>
<td>12.36, 19.05, 34.12</td>
</tr>
<tr>
<td>[Co(HL)(HL^1)SO4]2H2O</td>
<td>3397s</td>
<td>159s</td>
<td>576s</td>
<td>425s</td>
<td>371m</td>
<td>14.99, 23.87, 33.98</td>
</tr>
<tr>
<td>[Cu(HL)(HL^1)SO4]H2O</td>
<td>3376s</td>
<td>1309s</td>
<td>576s</td>
<td>422s</td>
<td>-</td>
<td>12.58, 23.92, 33.35</td>
</tr>
</tbody>
</table>

HL = Trimethoprim, HL^1 = Sulfamethoxazole, b = broad, s = strong, m= medium, w= weak; 1kK = 1000cm^-1

Solubility and Conductance Measurements: The molar conductances of the complexes were measured in DMSO because the complexes were insoluble in alcohols, nitromethane and methylene chloride. The values obtained were in the range 12.22- 19.90 Ω^-1 cm^2 mol^-1 indicating their covalent nature [23].

Electronic Spectra and Magnetic Moments: The ultraviolet spectra of the HL (Trimethoprim) and HL^1 (Sulfamethoxazole) were characterized by strong absorption maxima at 25.58 kK and 32.79 kK respectively assigned to n–π* and π–π* transitions. In the metal complexes, these bands shifted to 31.45 - 34.12 kK due to coordination (Table 2).

The Mn (II) complex showed two absorption bands at 17.24 kK and 23.92 kK typical of low spin octahedral geometry and was assigned to T^2g – A^2g and T^2g – B1g transitions respectively. The effective magnetic moments of high spin Mn(II) complexes are expected to be in the range 5.50-5.90 B.M while low spin octahedral Mn(II) have moments in the range 1.73-2.20 B.M. Consequently, a moment of 1.87 B.M. observed for this complex indicated that, it was low spin octahedral [24].

The Fe (II) complex had a single absorption band expectedly at 16.86 kK, typical of 6-coordinate, high spin octahedral geometry and was assigned to 5T^2g – 5E_g transition. An observed moment of 6.2 B.M for this complex was complimentary of high spin octahedral geometry [25].

The Co(II) complex exhibited three bands at 12.36, 19.05 and 23.42 kK, assigned to 4T^2g(F)–4T^2g(F), (v_1), 4T^2g(F)– 4A^2g(F), (v_1) and 4T^2g(F) – 4T^1g(F), (v_1), transitions of an octahedral geometry. An observed moment of 5.3 B.M was complimentary of this geometry since moments in the range 4.7-5.3 B.M is usually observed for octahedral Co (II) compounds [26].

338
The Ni(II) complex displayed two absorption bands, typical of an octahedral geometry, at 12.50 kaka and 23.98 kK and were assigned to $^{1}A_{1g}(F)$ and $^{3}T_{2g}(F)$ transitions respectively. Generally, square planar Ni(II) complexes are diamagnetic while octahedral and tetrahedral complexes are paramagnetic with moments in the range 2.90-3.40 B.M and 3.50-4.10 B.M respectively. The Ni(II) complex in this study had a moment of 3.38 B.M and hence octahedral [27, 28].

The Cu(II) complex exhibited two unsymmetrical absorption bands at 13.55 kK and 23.98 kK assigned to $^{2}B_{2g} ightarrow {^2}B_{1g}$ and $^{2}B_{2g} ightarrow {^2}E_{g}$ transitions of a tetragonal (octahedral) geometry [29]. A moment of 1.9-2.2 B.M. is usually observed for mononuclear copper(II) complexes, regardless of stereochemistry, expectedly higher than the spin only moment due to orbital contribution and spin-orbit coupling. The Cu(II) complex in this study, had a moment of 2.3 B. M complimentary of octahedral geometry [30, 31].

The Zn(II) complex showed only the CT transitions from M ? L, as no d-d transition is expected at 12.58 and 23.92 kK. This complex was expectedly diamagnetic and assumed a 6-coordinate octahedral geometry [32, 33].

**Infrared Spectra:** The relevant infrared data are presented in Table 2. The strong bands at 3376 cm$^{-1}$, 3298 cm$^{-1}$ and 3298 cm$^{-1}$ in TMP and SMX were assigned as $\nu(\text{NH})$ band [28]. On coordination, these bands shifted to 3382 - 3237 cm$^{-1}$ in the spectra of the complexes indicating the coordination of the amino group’s nitrogen to the metal(II) ions without deprotonation [31]. The azomethine, $\nu(C=\text{N})$, vibrations at 1635 and 1593 cm$^{-1}$ in TMX shifted to 1625 - 1615 cm$^{-1}$ and 1601 - 1595 cm$^{-1}$ in the metal complexes respectively, confirming the coordination of the imine nitrogen atom to the metal(II) ions [29, 34]. The sulfone bands at 1309 cm$^{-1}$ in SMX remains unshifted/ marginally shifted to 1312 cm$^{-1}$ in the complexes indicative of non-coordination of one of the sulphone oxygen atoms, whereas the second sulphone band at 1154 cm$^{-1}$ shifted to 1161 - 1146 cm$^{-1}$ in the metal complexes confirming coordination to the metal atom through the second sulfone oxygen atom [14]. The bands due to $\nu(M-\text{Cl})$, $\nu(M-\text{O})$ and $\nu(M-N)$ were observed in the range 395 - 23.26 kK and 23.92 kK. This complex was expectedly diamagnetic and confirm all the metal complexes and their ligands showed good activity against the clinical strain of E. coli with inhibitory zones range of 12.0 - 31.0 mm. Interestingly, all the metal complexes and their ligands had activity against Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus sp (environmental isolate) and E. coli (clinical strain) with inhibitory zones range of 19.0-30.0 mm, 6.0-23.0 mm, 10.0-17.0 mm and 12.0-26.0 mm respectively. In contrast, the metal complexes and their ligands had mostly higher antimicrobial activities than Streptomycin (22.0 mm, 20.0 mm, 12.0 mm and 19.0 mm) against Streplococcus sp,

**Table 3.0: Antimicrobial activities of the Ligands and their metal(II) complexes**

<table>
<thead>
<tr>
<th>Metal Complexes</th>
<th><a href="H2O">Mo(HL)</a>2</th>
<th><a href="H2O">Fe(HL)</a>2</th>
<th><a href="H2O">Co(HL)</a>2</th>
<th><a href="SO4">Ni(HL)(HL)2</a>2</th>
<th><a href="SO4">Cu(HL)(HL)2</a>2</th>
<th><a href="SO4">Zn(HL)(HL)2</a>2</th>
<th>HL</th>
<th>HL+ + Streptomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (Typed strain)</td>
<td>R</td>
<td>27.0±0</td>
<td>31.0±0</td>
<td>12.0±0</td>
<td>23.0±0</td>
<td>29.0±0</td>
<td>17.0±0</td>
<td>27.0±0</td>
</tr>
<tr>
<td>E. coli (Clinical strain)</td>
<td>26.0±0</td>
<td>27.0±0</td>
<td>31.0±0</td>
<td>12.0±0</td>
<td>23.0±0</td>
<td>29.0±0</td>
<td>17.0±0</td>
<td>27.0±0</td>
</tr>
<tr>
<td>Streptococcus sp (clinical/blood)</td>
<td>25.0±0</td>
<td>25.0±0</td>
<td>29.0±0</td>
<td>12.0±0</td>
<td>25.0±0</td>
<td>26.0±0</td>
<td>20.0±0</td>
<td>25.0±0</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>28.0±0</td>
<td>25.0±0</td>
<td>27.0±0</td>
<td>7.0±0</td>
<td>25.0±0</td>
<td>25.0±0</td>
<td>15.0±0</td>
<td>29.0±0</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>22.0±0</td>
<td>20.0±0</td>
<td>23.0±0</td>
<td>11.0±0</td>
<td>27.0±0</td>
<td>32.0±0</td>
<td>20.0±0</td>
<td>30.0±0</td>
</tr>
<tr>
<td>Salmonella sp</td>
<td>25.0±0</td>
<td>22.0±0</td>
<td>27.0±0</td>
<td>10.0±0</td>
<td>25.0±0</td>
<td>25.0±0</td>
<td>11.0±0</td>
<td>9.0±0</td>
</tr>
<tr>
<td>Bacillus sp (Food)</td>
<td>31.0±0</td>
<td>25.0±0</td>
<td>19.0±0</td>
<td>27.0±0</td>
<td>5.0±0</td>
<td>21.0±0</td>
<td>25.0±0</td>
<td>15.0±0</td>
</tr>
<tr>
<td>Staphylococcus sp</td>
<td>30.0±0</td>
<td>32.0±0</td>
<td>29.0±0</td>
<td>29.0±0</td>
<td>23.0±0</td>
<td>25.0±0</td>
<td>19.0±0</td>
<td>22.0±0</td>
</tr>
<tr>
<td>Pseudomonas sp (Clinical)</td>
<td>20.0±0</td>
<td>20.0±0</td>
<td>23.0±0</td>
<td>6.0±0</td>
<td>19.0±0</td>
<td>21.0±0</td>
<td>20.0±0</td>
<td>15.0±0</td>
</tr>
<tr>
<td>Pseudomonas sp (Environmental)</td>
<td>13.0±0</td>
<td>10.0±0</td>
<td>15.0±0</td>
<td>R</td>
<td>17.0±0</td>
<td>17.0±0</td>
<td>19.0±0</td>
<td>15.0±0</td>
</tr>
<tr>
<td>Bacillus sp (Environmental)</td>
<td>25.0±0</td>
<td>22.0±0</td>
<td>23.0±0</td>
<td>11.0±0</td>
<td>25.0±0</td>
<td>27.0±0</td>
<td>19.0±0</td>
<td>29.0±0</td>
</tr>
</tbody>
</table>

HL= Trimethoprim; HL = Sulfamethoxazole; R= Resistance, + = positive control.
P. mirabilis, C. albicans and Salmonella sp with inhibitory zones range of 25.0-29.0 mm, 25.0-29.0 mm, 20.0-32.0 mm and 22.0-27.0 mm respectively. The better activity of the metal complexes was due to chelation, which reduces the polarity of the metal atom and increases lipophilic character, favouring its permeation through lipid layers of the organism membrane while, the inactivity of some of the metal(II) complexes in this study may be attributed to their probable lipophobic nature and / microbe strain [35]. Conclusively, all the metal complexes and their ligands showed good to excellent activity against all the microbes used with the exception of the Ni(II) complex which was inactive against Pseudomonas sp (environmental). Thus, proving their potentials as broad-spectrum antimicrobial agents’ in-vitro.

CONCLUSION

The drugs, Trimethoprim (HL) and Sulfamethoxazole (HL'), formed only low spin, octahedral complex with the Mn(II) ion while it formed high spin octahedral complexes with Co, Ni, Cu, Fe, Zn ions. These complexes were all covalent, magnetically dilute and octahedral as corroborated by conductance measurements, room temperature magnetic moments, infrared and electronic spectroscopies. Interestingly, all the metal complexes showed broad-spectrum antimicrobial activities against all the microbes used with the exception of Ni(II) complex proving their potentials as broad spectrum antimicrobial agent.

REFERENCES


