SYNTHESIS, ANTIBACTERIAL AND TOXICOLOGY STUDY OF MN(II), CO(II) AND NI(II) METAL COMPLEXES OF SULFADOXINE MIXED WITH PYRIMETHAMINE

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ABSTRACT

Three mixed ligand metal complexes of Sulphadoxine and Pyrimethamine were prepared by using Mn(II), Ni(II) and Co(II) metal chloride hexahydrate and characterized by elemental analysis, molar conductivity, magnetic susceptibility measurement, AAS, IR and UV-Vis spectroscopy. Some physical parameters were obtained using molar conductance measurement and melting point determination. Based on the analytical and spectroscopic data, the complexes were proposed to have the formulae: [ML1L2](Cl)2 (where M = Mn(II), Ni(II) and Co(II); L1 = sulphadoxine, L2 = pyrimethamine). The spectroscopic data proposed that L1 and L2 coordinated through N of NH2 groups in L1 and through N atom of NH group in L2. Thus, pyramethamine was proposed to be a tridentate ligand, while sulphadoxine was proposed to be a monodentate ligand. Micro-analysis further supported the proposed structure for the complexes. The antibacterial activity of the metal complexes were compared with their ligands by screening them against isolates of some strains of (-) Escherichia coli, (+) Proteus sp., (+) Pseudomonas aureginosa and Salmonella typhi by using diffusion method. The results obtained showed the metal complexes to be more potent antibacterial than the parent drugs against the four species used. Toxicology tests against some tissues of albino rat (Rattus novergicus) revealed toxicity of the complexes in the kidney as compared to the parent drugs. However, ALP values for metal complexes were found to be non-significantly different from the ALP values obtained for livers and the sera. This indicates that the metal complexes are not excessively toxic.

Keywords: Metal complexes, complexation, antibiotics, antimicrobial properties, alkaline phosphatase.

INTRODUCTION

Vector borne infectious diseases are rapidly spreading in tropical and sub-tropical regions including parts of the America, Asia and Africa (Mendis et al., 2006). These diseases results in millions of death across the globe annually. The spread of infectious diseases is not just associated with poverty but also serve as major hindrance to economic development (Ajibade, 2008; Sachs and Malaney, 2002). Although, many chemotherapeutic agents are in the market, there is tremendous increase in the ability of the parasite to survive or multiply despite the administration and absorption of drugs. This is generally accepted to be initiated primarily through a spontaneous mutation that reduces level of sensitivity of the drug. Also, biological mechanism behind the resistance was subsequently reported to be related to the development of an efflux mechanism that expels the drug from the parasite before reaching the required concentration that will effectively inhibit the process of heme polymerization (Elzahany et al., 2008; Shiva et al., 2012). Antimicrobial resistance is fast becoming a global concern with rapid increase in multidrug resistance in bacteria. Thus, some previously treatable pathogens are now becoming untreatable. Pyramethamine is notably used in the treatment of chloroquine-resistant cases of malaria due to Plasmodium faciparum in combination with sulfadoxine (Trampuz et al., 2003). Compounds containing pyrimidine rings have been reported to possess biological activities (Morad et al., 2007). Many therapeutic agents contain pyrimidine ring which enable them to coordinate with metal ion in the body system. Resistance of P. faciparum to first line treatment drugs have become a major concern for developing countries. Therefore, it has become highly imperative to prepare possible new antibacterial agents that can serve as future replacement for the present crop of drugs. However, the medicinal uses and applications of metals complexes are of increasing clinical and commercial importance. The essential trace metals cannot be over emphasized in a living system. Transition metal ions are responsible for the proper functioning of different enzymes (Farrell, 2003; Roat-Malone, 2007). In our effort to search for novel chemotherapeutic drugs against parasitic diseases, we reported the synthesis, characterization, antimicrobial and toxicology study of Co(II), Mn(II) and Ni(II) complexes of pyramethamine mixed with sulfadoxine.
MATERIALS AND METHODS

Pyramethamine and sulfadoxine were obtained from Bond Chemicals, Lagos, Nigeria. They are products of Sigma Chemical Company, USA. All the solvents and other reagents were of high purity (Aldrich and Sigma products) and were used without further purification. CoCl2.6H2O, NiCl2.6H2O and MnCl2.6H2O were used as metal ion sources. Isolates of gram (-) *Escherichia coli*, gram (+) *Staphilococcus aureus*, gram (+) *Pseudomonas aureginosa* and gram (+) *Salmonella typhi* were obtained from Microbiology Department, Covenant University, Nigeria. Albino rats (*Rattus norvegicus*) obtained from Chemistry Department, University of Ilorin were used for toxicity study.

Experimental methods

IR spectra of the samples in KBr pellets were obtained in the ranges of 400 to 4000 cm⁻¹ on Thermo Nicolet FTIR spectrometer. Metal analyses were determined by AAS on Thermo S Series AAS. The analyses of carbon, hydrogen, oxygen and nitrogen were carried out on a Perkin–Elmer 204C micro analyzer, UV-Vis spectra were obtained on Thermo Genesys IOVV Scanning UV-Vis spectrometer. Magnetic moment was carried out by using Faraday balance. The melting point determination was carried out using Gallenkamp melting point apparatus. Conductivity measurement was carried out using CON 6/TDS6 Handheld Conductivity/TDS meter with DMF as solvent.

Synthesis of metal complexes

0.6206g (2mmol) of sulfadoxine and 0.5000g (2mmol) of pyrimethamine were dissolved in 20ml of ethanol separately (Martak et al., 2009). The solutions were mixed thoroughly together in round bottom flask. The resulting mixture was stirred under reflux for 1 h, after which 0.01 mol. of each of the metal salt in 20ml methanol was added. The reaction mixture was refluxed for 3 h, after which the solution was allowed to cool to room temperature and left on the bench for 2 weeks. The crystals formed were filtered under vacuum, washed twice with ethanol and dried in desiccator containing CaCl₂ as drying agent. Purity of the compounds was confirmed by using thin layer chromatography (TLC).

Antimicrobial study

The inhibitory activity of the ligands and the metal complexes were determined by screening their antibacterial activity against pathogenic bacterial species like gram (-) *E. coli*, gram (+) *S. aureus*, gram (+) *P. aureginosa* and gram (+) *S. typhi* (Collins and Lyne, 1980; Garba and Salihu, 2011). Isolates of the bacteria were cultured in nutrient broth and incubated at 37°C for 24h. Sulphadoxine and pyramethamine were used as standard while only methanol was used as control. Nutrient agar (5g nutrient broth; 3.1g of nutrient agar in 200ml of sterile water for 8 plates) was prepared as the basal medium for the cultured bacteria and autoclaved. 1.0cm diameter wells were punched and 0.1ml of sterile solutions of each of the compounds (1.0w/v) was applied to each well and incubated at 37.1°C for one to three days. The observed zone of inhibition (in mm) is presented as mean ± SEM in figures 1 to 4.

\[
\text{% Inhibition} = \frac{\text{Average diameter of bacterial colony on the test plate (mm)}}{\text{Average diameter of growth of bacterial colony on the control plate (mm)}} \times 100
\]

Toxicology study

In order to compare the level of toxicity of the metal complexes with the ligands, 30 albinos rats weighing between 150 and 170g were used for toxicity study (Ogunniran et al., 2007, 2008). Enzyme activity (ALP) and protein concentration in the livers, kidneys and sera were determined as described by Tella and Obaleye (2010).

Statistical analysis

Statistical significance was determined using Duncan multiple range test and the values were considered statistically significant at P< 0.05.

RESULTS AND DISCUSSION

Physical characteristics and micro analytical data of the ligands and complexes are given in table 1. The analytical data confirmed the proposed formula of the complexes. The results of C, H, O and N percentage are in accordance with the composition suggested for most of the complexes. This is supported by the results of metal content analysis, which correlate with the calculated values. The presence of chloride ion inside the coordination sphere was confirmed by lack of white precipitate of AgCl with the use of AgNO₃ solution (Vogel, 1989). Hence, the proposed synthetic equation for the synthesized complexes could be represented as:

\[
\text{MCl}_2.6\text{H}_2\text{O} + \text{L}_1 + \text{L}_2 \rightarrow \text{ML}_1\text{L}_2\text{Cl}_2 + 6\text{H}_2\text{O}
\]

Where M = Mn(II), Co(II) and Ni(II), L₁ = sulphadoxine, L₂ = pyrimethamine

The complexes were found to be soluble in methanol and slightly soluble in acetone, chloroform, dimethylformamide, dimethysulfoxide and benzene. However, they were found to be non-soluble in distilled water, ethanol, n-haxane and display good stability in air at room temperature. The molar conductance of 10⁻³ M solutions of the ligands and metal complexes in DMF are in the range 12.37 to 19.29 Ωcmmol⁻¹ indicating their non-electrolytic nature (Vogel, 1989). The complexes are of average percentage yield range of 44 to 58 which indicate that they can be synthesized commercially.
complexes were found to possess higher melting point as compared to the ligands. The increase in melting point is attributed to increase in molecular weight of the complexes due to coordination of metal ions to the ligands (McCleverty and Meyer, 2003).

Infrared spectra
The infra-red spectra of the ligands were compared with those of the metal complexes (Table 2). They showed similar bands as expected. The strong band in the region 3682 to 3600 cm$^{-1}$ in sulfadoxine spectrum assigned to $\nu$(N-H) (Salisu et al., 2009; Vogel, 1989; Fessenden and Fessenden, 1990) also, it appeared as strong bands at 3600 and at 3340 cm$^{-1}$ in pyrimethamine spectrum. Similar bands were observed in metal complexes at lower wavelengths coupled with reduction in intensities. The observations have been attributed to coordination of the vibrational group to the central metal ion (Nora, 2011). This probably account for reduction in intensities of the bands. The assignment is supported by (C-N) bending vibration observed as medium band at 1170 and 1080 cm$^{-1}$ in sulphadoxine and pyrimethamine spectrum, respectively (Watson, 2000). The band has shifted to higher wavelength in the spectra of metal complexes with reduction in intensity. Other bands assigned to vibrational groups like $\nu$(C=C), $\nu$(S=O) and $\nu$(C-H) in the ligands were also observed with shift in their region of absorption in the spectra of metal complexes as a result of effect of complexation on them. However, $M\rightarrow L$ bands which were found in the range of 500 to 780 cm$^{-1}$ in the spectra of metal complexes were conspicuously absent in the spectra of the ligands (Obaleye et al., 2001; Ajibade, 2008).

Electronic spectral and magnetic studies
The electronic spectra of sulphadoxine (Table 3) showed two absorption bands at 49505 and 36900 cm$^{-1}$. The bands were assigned to $\pi-\pi^*$ of the phenyl rings in the ligand.

Table 1. Colour, decomposition temperature, conductivities and analytical data of the ligands L$_1$ and L$_2$ and their mixed ligands metal complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>% C found (Calc)</th>
<th>% H found (Calc)</th>
<th>% O found (Calc)</th>
<th>% N found (Calc.)</th>
<th>Metal found (Calc)</th>
<th>Conductivity $\Omega^{-1}$ cm$^2$ mol$^{-1}$</th>
<th>M.Pt. °C</th>
<th>Colour/state</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfadoxine (L$_1$)</td>
<td>46.44</td>
<td>46.17</td>
<td>4.22</td>
<td>4.01</td>
<td>20.62</td>
<td>20.32</td>
<td>18.06</td>
<td>18.03</td>
<td>-</td>
</tr>
<tr>
<td>Pyramethamine (L$_2$)</td>
<td>57.95</td>
<td>57.89</td>
<td>4.46</td>
<td>4.44</td>
<td>-</td>
<td>22.52</td>
<td>22.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mn(L$_1$L$_2$)Cl$_2$</td>
<td>42.08</td>
<td>42.01</td>
<td>3.52</td>
<td>3.44</td>
<td>9.34</td>
<td>9.30</td>
<td>16.36</td>
<td>16.11</td>
<td>14.55</td>
</tr>
<tr>
<td>Co(L$_1$L$_2$)Cl$_2$</td>
<td>41.84</td>
<td>41.32</td>
<td>3.51</td>
<td>3.49</td>
<td>9.29</td>
<td>9.22</td>
<td>16.26</td>
<td>16.17</td>
<td>12.37</td>
</tr>
<tr>
<td>Ni(L$_1$L$_2$)Cl$_2$</td>
<td>41.86</td>
<td>41.81</td>
<td>3.51</td>
<td>3.48</td>
<td>9.29</td>
<td>9.27</td>
<td>16.27</td>
<td>16.22</td>
<td>12.49</td>
</tr>
</tbody>
</table>

Table 2. IR Spectra (4000-400 cm$^{-1}$) of the ligands L$_1$ and L$_2$ and their mixed metal complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\nu$(N-H) cm$^{-1}$</th>
<th>$\nu$(C-H) cm$^{-1}$</th>
<th>$\nu$(C=C) cm$^{-1}$</th>
<th>$\nu$(C-N) cm$^{-1}$</th>
<th>$\nu$(C=O) cm$^{-1}$</th>
<th>$\nu$(S=O) cm$^{-1}$</th>
<th>M→L</th>
</tr>
</thead>
<tbody>
<tr>
<td>L$_1$</td>
<td>3682 s 3600 s</td>
<td>3010s,b</td>
<td>1580 s</td>
<td>1170 s</td>
<td>1210 s,b</td>
<td>1190 s</td>
<td>-</td>
</tr>
<tr>
<td>L$_2$</td>
<td>3605 s 3410 s</td>
<td>3010s,b</td>
<td>1600 s,1510 s</td>
<td>1080 m</td>
<td>1220 s,b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mn(L$_1$L$_2$)Cl$_2$</td>
<td>3620 m 3340 m</td>
<td>3005.01s,b</td>
<td>1605 s,1510 s</td>
<td>1300 s</td>
<td>1430 s</td>
<td>1250 s,1200 s,b</td>
<td>500 s</td>
</tr>
<tr>
<td>Co(L$_1$L$_2$)Cl$_2$</td>
<td>3602 m 3340 m</td>
<td>3005.01s,b</td>
<td>1560 s,1510 m</td>
<td>1310 s</td>
<td>1310 w</td>
<td>1225 s,1200 s,b</td>
<td>500 m</td>
</tr>
<tr>
<td>Ni(L$_1$L$_2$)Cl$_2$</td>
<td>3600 s 3405 m,b</td>
<td>3105.02s,b</td>
<td>1600 s,1520 m</td>
<td>1410 s</td>
<td>1410 s</td>
<td>1200 s,1200 s,b</td>
<td>780 vs</td>
</tr>
</tbody>
</table>

w- Weak, s- strong, m- medium, vw- very weak, vs-very strong, m,b- medium and broad, s,b- strong and broad.
due to conjugation (Vogel, 1989). Similar bands were observed at 49505 and 34965 cm\(^{-1}\) in the pyrimethamine spectrum. However, these bands were observed in the metal complexes to have shifted to higher wavelength due to complexation (Ajibola et al., 1998). Extra bands observed in Mn(II), Co(II) and Ni(II) complexes were attributed to d-d transition. The intense green colour of Ni(II) complex showed three weak absorption bands at 24272, 25907 and 26810 cm\(^{-1}\) due to the splitting of ground 3F term and the presence of the 3P term. The absorption bands were attributed to \(^3\)A\(_{2g}(F)\rightarrow\(^3\)T\(_{2g}(F)\), \(^3\)A\(_{2g}(F)\rightarrow\(^3\)T\(_{2g}(F)\) and \(^3\)A\(_{2g}(F)\rightarrow\(^3\)T\(_{1g}(P)\) respectively. The broad peak absorption bands observed indicate large distortion from octahedral symmetry, as a result of which unsymmetrical bands were observed. However, magnetic moment value of 3.17 BM obtained for the complex is within the octahedral environment range of 2.8-3.5 BM. Thus, the complex could be distorted octahedral (Cotton and Wilkinson, 1985). The electronic spectra of Co (II) complex displayed three absorption bands assigned to \(^5\)T\(_{1g}(F)\rightarrow\(^3\)T\(_{2g}\), \(^5\)T\(_{1g}(F)\rightarrow\(^3\)T\(_{1g}(P)\) and \(^5\)T\(_{1g}(F)\rightarrow\(^3\)A\(_{2g}\) transitions respectively. These bands are characteristics of high spin octahedral Co(II) complex. The magnetic measurement of Co(II) complex exhibited magnetic moment value of 4.64 BM which is within the octahedral range of 4.3 - 5.2 BM. Mn(II) complex showed absorption bands at 20576, 25510 and 34722 cm\(^{-1}\). The bands were assigned to \(^6\)A\(_{1g}\rightarrow\(^4\)T\(_{1g}\), \(^6\)A\(_{1g}\rightarrow\(^4\)T\(_{2g}(G)\) and \(^6\)A\(_{1g}\rightarrow\(^4\)T\(_{2g}(D)\) transitions respectively. The complex exhibit magnetic moment of 5.82 BM, which supported octahedral geometry around Mn (II) ion (Fahmideh et al., 2010).

Table 3. UV-Vis. Spectra assignments of sulphadoxine, pyramethamine and their mixed metal complexes and magnetic moment measurements of the metal complexes.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Wavelength (nm)</th>
<th>(cm(^{-1}))</th>
<th>Assignment</th>
<th>Magnetic moment (BM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L(_1)</td>
<td>202</td>
<td>49505</td>
<td>(\pi-\pi^*)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>271</td>
<td>36900</td>
<td>(n-\pi^*)</td>
<td></td>
</tr>
<tr>
<td>L(_2)</td>
<td>202</td>
<td>49505</td>
<td>(\pi-\pi^*)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>286</td>
<td>34965</td>
<td>(n-\pi^*)</td>
<td></td>
</tr>
<tr>
<td>Mn(L(_1)L(_2)Cl(_2))</td>
<td>486</td>
<td>20576</td>
<td>(^6)A(<em>{1g})\rightarrow(^4)T(</em>{1g})</td>
<td>5.82</td>
</tr>
<tr>
<td></td>
<td>392</td>
<td>25510</td>
<td>(^6)A(<em>{1g})\rightarrow(^4)T(</em>{2g}(G))</td>
<td></td>
</tr>
<tr>
<td></td>
<td>288</td>
<td>34722</td>
<td>(^6)A(<em>{1g})\rightarrow(^4)T(</em>{2g}(D))</td>
<td></td>
</tr>
<tr>
<td>Co(L(_1)L(_2)Cl(_2))</td>
<td>443</td>
<td>22573</td>
<td>(^3)T(<em>{1g}(F)\rightarrow(^3)T(</em>{2g})</td>
<td>4.64</td>
</tr>
<tr>
<td></td>
<td>317</td>
<td>31546</td>
<td>(^3)T(<em>{1g}(F)\rightarrow(^3)T(</em>{1g}(P))</td>
<td></td>
</tr>
<tr>
<td></td>
<td>342</td>
<td>29240</td>
<td>(^3)T(<em>{1g}(F)\rightarrow(^3)A(</em>{2g})</td>
<td></td>
</tr>
<tr>
<td>Ni(L(_1)L(_2)Cl(_2))</td>
<td>412</td>
<td>24272</td>
<td>(^3)A(<em>{2g}(F)\rightarrow(^3)T(</em>{2g}(F))</td>
<td>3.17</td>
</tr>
<tr>
<td></td>
<td>386</td>
<td>25907</td>
<td>(^3)A(<em>{2g}(F)\rightarrow(^3)T(</em>{2g}(F))</td>
<td></td>
</tr>
<tr>
<td></td>
<td>373</td>
<td>26810</td>
<td>(^3)A(<em>{2g}(F)\rightarrow(^3)T(</em>{1g}(P))</td>
<td></td>
</tr>
</tbody>
</table>

Structure 1. The proposed structure of the prepared complexes.
Suggested structural formulae of the complexes
From the spectral data and the elemental analyses, the proposed structure of the prepared complexes is as shown in Structure 1.

Biological study
In vitro antibacterial activities of the ligands were compared graphically to those of the mixed ligands metal complexes (Figs. 1 to 4). From the results obtained, it can be concluded convincingly that the metal complexes are more active than the ligands against all the bacteria used. Increase in activity observed could be attributed to coordination of the ligands to metal ions. However, Ni(L₁L₂)Cl₂ was found to be less active against g(+) S. typhi (Fig. 4). This observation could be as a result of ability of the bacterium to develop efflux mechanism against the complex (Barbara et al., 2010; Ajibade, 2008). It could also be as a result of effect of coordination on the active site in the complex (Heslop and Jones, 1986). The overall results confirmed that the metal complexes are more potent than the parent ligands under identical experimental conditions. This would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy's chelation theory (William, 1981).

Results of toxicology assay
The values of ALP activities in the kidney, liver and serum following the administration of ligands and their mixed metal complexes as compared to the control are
shown in figures 5 to 7. The results indicated that kidney (Fig. 5) produced significant increase (P<0.05) in ALP activities as compared to the control value. Sulp and pyra produced non-significant difference as compared to control value. This is an indication that increase in their enzyme activity did not lead to damages to plasma membrane of the organ (Ogunniran et al., 2007). However, administration of the metal complexes increased the enzyme activity beyond tolerance level (75.62 to 83.79 nM/min/mg protein) in the kidney and thus produced significantly different values as compared to control value. The trend confirmed alteration in the enzymes activity of the kidneys which may likely cause damage to their plasma membrane (Yakubu et al., 2005). It may also be attributed to induction in enzyme synthesis which leads to hydrolysis of phosphate ester of the organ and other essential cells (Roat-Malone, 2007). The damages of the tissue cell plasma membrane may lead to leakages of membrane components into the extracellular fluid (Yakubu et al., 2005). The ALP activities values for sera and livers (Figs. 6 and 7, respectively) of the rats administered with metal complexes were found to be non-significantly (P<0.05) different from control values. The non-significant changes observed in ALP activity of the liver and serum suggested partial non-damage effect of the metal complexes in them. It can therefore be concluded that even though the metal complexes are toxic than the parent ligands, they can still be tolerated in the body system.

Fig. 3. Zone of inhibition (%) of the ligands and metal complexes against *P. aureginose*.

Fig. 4. Zone of inhibition (%) of the ligands and metal complexes against *S. typhi*. 
CONCLUSION

This study shows feasibility and justification for synthesis of mixed antibiotics metal complexes using sulphadoxine and pyramethamine as ligands. The complexes possessed better physical properties and are more effective as chemotherapy agents than their parent antibiotics. However, the complexes are toxic at the dose level used to the kidneys but not to livers and sera of the rat administered with the complexes. Thus, the present study concluded that complexes could be used as good drug of choice to manage the bacterial diseases after evaluating the in vivo effect of metal complex on experimental higher animal and clinical trials.

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