SYNTHESIS AND PHARMACOLOGICAL SCREENING OF SCHIFF’S BASE METAL COMPLEXES OF SULPHANILAMIDE

K. Hariprasath*1 and I. Sudheer babu1

*1Sir C. R. Reddy College of Pharmaceutical Sciences, Eluru, West Godavari district, Andhra Pradesh, India.

ABSTRACT
Ninety elements occur naturally on earth. Out of these, nine are radioactive and among the remaining eighty one that could support life, sixty one are metals. Our bodies are 3% metal. Researchers have been established that some of metal complexes were biologically active than their parent ligands. Sulphanilamide is a well known antimicrobial agent, which are widely used in various diseases. Schiff’s bases of Sulphanilamide was prepared by treating with aromatic aldehyde like para diethyl amino benzyldehyde and paradinetheryl amino benzyldehyde and their metal complexes were synthesized by reacting with metals like copper, zinc and cadmium. The metal complexes were characterized by elemental analysis, IR and H1NMR analysis. The in vitro antimicrobial and antioxidant activity were performed. The copper metal complexes of sulphanilamide possess good antimicrobial activity, whereas both copper and zinc metal complexes possess excellent antioxidant activity.

KEYWORDS
Radioactive, Metal complexes, Sulphanilamide, Schiff’s bases, Antimicrobial and Antioxidant activity.

INTRODUCTION
Schiff bases are the condensation products of primary amines with carbonyl compounds. The common structural features of this compound is the azomethine group with a general formula RCHC=NR1 where R and R1 are alkyl, aryl, cycloalkyl or heterocyclic groups. Schiff bases are the important compounds in the field of medicinal chemistry owing to their wide range of biological activities and industrial applications. Metal complex is a structure consisting of a central atom (or) ion (metal) bonded with anions (ligands). The
microorganisms adsorb metal ions on their cell walls and as a result respiration processes of cells are disturbed and protein synthesis is blocked which is the requirement for further growth of organisms. The growth inhibition effects of metal ions are considerable. The proliferation of microorganisms is further restricted because the penetration of complexes in lipid membranes is facilitated by increased lipophilicity. The free radicals and reactive oxygen species (ROS) are involved in complete damage of our tissues and such type of damage can be avoided by employing antioxidants. Metal complexes possess considerable antioxidant activity by inhibiting the generation of reactive oxygen species. Literature review reveals that synthesized Schiff’s base metal complexes possess good pharmacological property. Sulpha drugs are well known antimicrobial agents, which are widely used in various diseases. So far sulpha drug metal complex has not been synthesized. Hence we aimed to synthesize Schiff’s base of sulphanilamide with aromatic aldehyde like para diethyl amino benzyldehyde and paradimethyl amino benzyldehyde and to form metal complexes above Schiff’s base with metals like copper, zinc and cadmium.

**MATERIALS AND METHODS**

**Synthesis of Schiff’s base**
The Schiff’s base has been synthesized by refluxing the reaction mixture of hot ethanolic solution (30 ml) of sulphanilamide (0.01 mole) with hot ethanolic solution (30 ml) of different aromatic aldehyde, like para diethyl amino benzyldehyde and paradimethyl amino benzyldehyde for about 2-3 hours at 60-70°C. The resulting solution was concentrated upto 10 ml on a water bath. The mixture was allowed to stand overnight. After that the colored solid product was filtered off, re-crystallized with ethanol and finally washed with petroleum ether (Figure No.1). The final product was dried under reduced pressure over anhydrous calcium chloride. The purity of synthesized compound was monitored by TLC using silica gel-G (yield 60-70%) of

**Synthesis of cationic derivative of Schiff base**
Cationic derivatives of Schiff bases were obtained by direct reaction between equimolar amount of the synthesized Schiff bases and methyl iodide in 50ml ethanol. The reaction mixture was refluxed for 8 hours and left overnight (Figure No.2). The precipitated products were filtered and recrystallized.

**Synthesis of transition metal complexes**
Metal ion solutions of anhydrous CuCl₂, ZnCl₂ and CdCl₂ (0.0005 mol) in 50ml ethanol was added with synthesized cationic derivative of Schiff base separately and refluxed for 6 hours. The reaction mixture was left overnight to complete the precipitation of the products (Figure No.3). The products were recrystallized with ethanol to obtain pure products. The metal complexes were characterized by elemental analysis, IR and H1NMR analysis.

**Anti bacterial activity**
Required number of Muller agar plates were prepared and divided into number of quadrant. Then the plates were inoculated with known test organism. Sterile discs were placed within each quadrant. Using micro pipette 10 micro liter of saturated solution of the derivatives is applied on the respective discs. Then the plates are incubated at 37°C for 18 to 24 hrs. After incubation for each derivative against different organisms was measured and tabulated.

**Antioxidant activity**
Hydrogen donating ability of extract was examined in the presence of DPPH stable radical. Sample stock solutions were diluted to final concentration of 0.5 - 5 gm/ml in ethanol.1ml of a 0.3mM DPPH solution of different concentration and allowed to react at room temperature. After 30minutes the absorbance values were measured at 518 nm and converted in to percentage antioxidant activity using the following formula

\[ AA\% = 100 - \left( \frac{Abs_{\text{sample}} - Abs_{\text{blank}} \times 100}{Abs_{\text{control}}} \right) \]

Ethanol (1.0 ml) plus plant extract solution was used as blank. DPPH solution (1.0 ml; 0.3mM) plus ethanol (2.5 ml) was used as negative control. The
positive controls were those using the standard solution. The EC_{50} values were calculated linear regression of plots where the abscissa represented the concentration of tested plant extract and the ordinate the average percentage of antioxidant activity from three separate tests^{12-14}.

RESULTS AND DISCUSSIONS
Characterization of synthesized compounds

1A1 Copper metal complex of (E) - N-(4-(diethyl, methyl amino) benzylidene)-(4-sulfonamidyl) benzenamine. M.F: C_{18}H_{24}Cl_{2}CuI_{2}N_{3}O_{2}S. M.wt: 732.73. IR (KBr) cm\(^{-1}\): NH bond stretching at 3400 cm\(^{-1}\), C=N bond stretching at 1690 cm\(^{-1}\), S=O stretching at 1140 cm\(^{-1}\), C=C stretching at 1600 and 1475 cm\(^{-1}\). H\(^{1}\) NMR (CDCl\(_3\)) \(\delta\) values: Multiplet at 7.44-7.9 for aromatic nucleus, singlet at 8.63 for N=CH peak, singlet at 2.49 for NH\(_2\) peak, two triplet at 3.34 for two CH\(_2\) groups in N-ethyl substitution, singlet at 3.34 for N-methyl substitution. Elem Anal Calc: C, 29.42; H, 3.29; Cl, 9.65; Cu, 8.65; I, 35.54; N, 5.72; O, 4.34; S, 4.36. Elem Anal Found: C, 29.42; H, 3.34; Cl, 9.65; Cu, 8.65; I, 35.54; N, 5.72; O, 4.36; S, 4.38; Zn, 3.98.

1A2- Zinc metal complex of (E) - N-(4-(diethyl, methyl amino) benzylidene)-(4-sulfonamidyl) benzenamine. M.F: C_{18}H_{24}Cl_{2}CuI_{2}N_{3}O_{2}Szn. M.wt: 706.7. IR (KBr) cm\(^{-1}\): NH bond stretching at 3400 cm\(^{-1}\), C=N bond stretching at 1690 cm\(^{-1}\), S=O stretching at 1140 cm\(^{-1}\), C=C stretching at 1600 and 1475 cm\(^{-1}\). H\(^{1}\) NMR (CDCl\(_3\)) \(\delta\) values: Multiplet at 7.44-7.9 for aromatic nucleus, singlet at 8.53 for N=CH peak, singlet at 2.49 for NH\(_2\) peak, three singlet peak at 3.00 for three N-methyl groups. Elem Anal Calc: C, 27.12; H, 2.85; Cl, 10.03; Cu, 8.99; I, 35.92; N, 5.95; O, 4.53; S, 4.54. Elem Anal Found: C, 27.29; H, 2.65; Cl, 10.13; Cu, 8.89; I, 35.62; N, 5.95; O, 4.83; S, 4.64.

1B1- Copper metal complex of (E) - N-(4-(trimethyl amino) benzylidene)-(4-sulfonamidyl) benzenamine. M.F: C_{10}H_{20}Cl_{2}CuI_{2}N_{3}O_{2}S. M.wt: 706.7. IR (KBr) cm\(^{-1}\): NH bond stretching at 3400 cm\(^{-1}\), C=N bond stretching at 1690 cm\(^{-1}\), S=O stretching at 1140 cm\(^{-1}\), C=C stretching at 1600 and 1475 cm\(^{-1}\). H\(^{1}\) NMR (CDCl\(_3\)) \(\delta\) values: Multiplet at 7.44-7.9 for aromatic nucleus, singlet at 8.36 for N=CH peak, singlet at 2.49 for NH\(_2\) peak, three singlet peak at 3.00 for three N-methyl groups. Elem Anal Calc: C, 27.12; H, 2.85; Cl, 10.03; Cu, 8.99; I, 35.92; N, 5.95; O, 4.53; S, 4.54. Elem Anal Found: C, 27.29; H, 2.65; Cl, 10.13; Cu, 8.89; I, 35.62; N, 5.95; O, 4.83; S, 4.64.

1B2- Zinc metal complex of (E) - N-(4-(trimethyl amino) benzylidene)-(4-sulfonamidyl) benzenamine. M.F: C_{10}H_{20}Cl_{2}CuI_{2}N_{3}O_{2}Szn. M.wt: 708.5. IR (KBr) cm\(^{-1}\): NH bond stretching at 3400 cm\(^{-1}\), C=N bond stretching at 1690 cm\(^{-1}\), S=O stretching at 1140 cm\(^{-1}\), C=C stretching at 1600 and 1475 cm\(^{-1}\). H\(^{1}\) NMR (CDCl\(_3\)) \(\delta\) values: Multiplet at 7.44-7.9 for aromatic nucleus, singlet at 8.11 for N=CH peak, singlet at 2.49 for NH\(_2\) peak, three singlet peak at 3.00 for three N-methyl groups. Elem Anal Calc: C, 27.12; H, 2.85; Cl, 10.01; Cu, 8.99; I, 35.92; N, 5.95; O, 4.53; S, 4.54. Elem Anal Found: C, 27.29; H, 2.65; Cl, 10.13; Cu, 8.89; I, 35.62; N, 5.95; O, 4.83; S, 4.64.
nucleus, singlet at 8.53 for N=CH peak, singlet at 2.49 for NH$_2$ peak, three singlet peak at 3.01 for three N-methyl groups. Elem Anal Calc: C, 25.43; H, 2.67; Cd, 14.88; Cl, 9.38; I, 3.359; N, 5.56; O, 4.24; S, 4.24. Elem Anal Found: C, 25.33; H, 2.47; Cd, 14.68; Cl, 9.78; I, 3.329; N, 5.88; O, 4.14; S, 4.24.

**Antibacterial activity**

Antibacterial activity was carried out by disc plate method using *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* microorganism. The results are given in the Table No.1. The copper metal complexes 1A1 and 1B1 showed potent antibacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa* and less antibacterial activity against *Escherichia coli* strains. This may be due to the higher stability of Cu complex than the Cd and Zn complex. The microorganisms adsorb metal ions on their cell walls and as a result respiration processes of cells are disturbed and protein synthesis is blocked which is the requirement for further growth of organisms$^{15,16}$.

**Antioxidant activity**

Antioxidant activity was carried out by DPPH method using ascorbic acid as standard. The results are given in the Table-2. The Copper and Zinc metal complexes of Schiff bases of sulpha drugs 1A1, 1A2, 1B1, 1B2 showed potent antioxidant activity when compared with IC$_{50}$ value 2.86µg/ml of standard ascorbic acid. Whereas the metal complexes of cadmium showed antioxidant activity but it is not upto the considerable level. The Schiff bases act as bidentate ligand coordinating through two azomethine nitrogen atoms. The marked antioxidant activity of complexes, in comparison to standard could be due to the coordination of metal with the condensed ring system, increasing its capacity to stabilize unpaired electrons and, thereby, to scavenge free radicals$^{17,18}$.

**Table No.1: Results of antibacterial activity of metal complexes of Schiff’s base of sulphanilamide**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compounds</th>
<th>Zone of inhibition (mm)</th>
<th>Bacillus cereus</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
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<tr>
<td>1</td>
<td>1A1</td>
<td>25</td>
<td>22</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1A2</td>
<td>21</td>
<td>17</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1A3</td>
<td>20</td>
<td>20</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1B1</td>
<td>16</td>
<td>19</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1B2</td>
<td>20</td>
<td>21</td>
<td>17</td>
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<tr>
<td>6</td>
<td>1B3</td>
<td>18</td>
<td>17</td>
<td>18</td>
<td></td>
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<tr>
<td>7</td>
<td>Streptomycin</td>
<td>24</td>
<td>24</td>
<td>21</td>
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Table No.2: Results of *in vitro* antioxidant activity of Schiff base metal complex of Sulphanilamide

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration µgm/ml</th>
<th>1A1</th>
<th>1A2</th>
<th>1A3</th>
<th>1B1</th>
<th>1B2</th>
<th>1B3</th>
<th>Standard (Ascorbic Acid) µgm/ml</th>
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<tr>
<td>1</td>
<td>0.1</td>
<td>12.14±0.52</td>
<td>11.14±0.52</td>
<td>10.14±0.56</td>
<td>22.14±0.62</td>
<td>12.14±0.33</td>
<td>08.14±0.52</td>
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<tr>
<td>2</td>
<td>0.5</td>
<td>15.48±0.88</td>
<td>13.48±0.88</td>
<td>13.48±0.68</td>
<td>35.48±0.38</td>
<td>15.48±1.88</td>
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<tr>
<td>3</td>
<td>1.0</td>
<td>20.12±1.02</td>
<td>22.12±1.02</td>
<td>18.12±1.00</td>
<td>44.12±1.02</td>
<td>20.12±1.68</td>
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<tr>
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<td>2.86</td>
</tr>
<tr>
<td>9</td>
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<td>90.31±0.08</td>
<td>88.31±0.08</td>
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<td>91.31±0.28</td>
<td>47.31±0.08</td>
<td>2.86</td>
</tr>
<tr>
<td>10</td>
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<td>94.50±1.28</td>
<td>91.50±1.28</td>
<td>92.50±1.28</td>
<td>92.50±1.28</td>
<td>94.50±1.34</td>
<td>49.50±1.28</td>
<td>2.86</td>
</tr>
</tbody>
</table>

![Scheme-1](image.jpg)

Compound 1A  \( R\text{-C}_2\text{H}_5, R_1\text{-C}_2\text{H}_5 \),  Compound 1B \( R\text{-CH}_3, R_1\text{-CH}_3 \)

**Figure No.1:** Scheme-1 (Synthesis of Sulphanilamide Schiff base)
Figure No.2: Scheme 2 (Synthesis of cationic Schiff base)

Figure No.3: Scheme 3 (Synthesis of cationic Schiff base metal complex)
CONCLUSION
Schiff’s base metal complexes provide a versatile platform for deriving various pharmacologically active drug and still more research is needed in this area to give a better drug molecule for future mankind. Future research in molecular receptor level is needed to elicit the mechanism of action.

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BIBLIOGRAPHY

