Synthesis, characterization and biological assay of Organotin derivatives of Sulphanilamide

Khan MA1, Akhtar S1, Shahid K1

ABSTRACT

Metals and its compound had played a major role as therapeutic agents in history of medicine and in modern pharmacology as well. A family of Organotin complexes (Sn⁴ - Sn⁶) of sulphanilamide were synthesized by the reaction of triorganotin or diorganotin chloride with the sulphanilamide by adding triethylamine in dry toluene. All the synthesized Complexes were characterized by combination of different techniques using Fourier Transform-Infrared spectroscopy, ¹H and ¹³C Nuclear magnetic resonance spectroscopy and elemental analysis. In-vitro antibacterial and antifungal activities were investigated. Biological screening showed that most of the derived complexes have significant activity against different tested pathogenic strains of bacteria and fungi.

INTRODUCTION

Organotin compounds have several applications in many fields from many years. Organotin(IV) complexes have been the subject of interest form the last few decades because of their biomedical and commercial applications. It has been observed that several organotin complexes are effective antifouling, antimicrobial, and antiviral agents. The interesting application of metal complexes in the treatment of numerous human diseases is a vigorously expanding area in biomedical and inorganic chemistry. The variation in coordination number, geometries, accessible redox states, thermodynamic, and kinetic characteristics and the intrinsic properties of the metal ion are some special characteristics of organometallic complexes that offer the medicinal chemists to employ different strategies for their exploitation. Their use in cancer chemotherapy is gaining mounting importance after the discovery of metal based drug Cisplatin. Sulphanilamide is a potent organic antibiotic agent consisting of an aniline derivitized with a sulphonamide group. Sulphanilamide competitively inhibits enzymatic reactions involving para- amino benzoic acid (PABA). PABA is involved in enzymatic reactions that produce folic acid which acts as a coenzyme in the synthesis of purine, pyrimidine and other amino acids. Sulphanilamide was used in World War 2 as a first-aid treatment to reduce infection rates and contributed to a dramatic reduction in mortality rates compared to previous wars. Modern antibiotics have supplanted sulphanilamide on the battlefield; however, sulphanilamide remains in use for treatment of vaginal yeast infections. Present study involves the synthesis, spectroscopic characterization, elemental analysis and biological screening of the newly synthesized organotin complexes of sulphanilamide.

MATERIALS AND METHODS

All the chemicals, metal salts, reagents were purchased from Sigma Aldrich laboratories and were used as purchased except toluene which was dried by using sodium wire prior to use. All the glassware were properly dried at 120°C. Synthesis was done at Riphah Institute of Pharmaceutical Sciences. Biological screening was done at Quaid-e-Azam University Pakistan. Spectroscopic characterization was done at Institute of Pharmaceutical Sciences, Kings Collage London.
General Procedure for the Synthesis of Organotin Derivatives of Sulphanilamide

Sulphanilamide was suspended in dry toluene (50 mL) and treated with triethylamine Et3N. The mixture was refluxed for 3 hours. To a solution triorganotin chloride or Diorganotin dichloride was added as solid to a reaction flask with constant stirring and the reaction mixture refluxed 3 hours. The reaction mixture contains Et3NHCl is filtered off such that filtrate had the organotin derivative. The solvent is removed through rotary apparatus. The mass left behind will be recrystallization from CHCl3,10-14 [9-13].

![Diagram of Synthesis Scheme](image)

**Figure 1:** Scheme for Synthesis of Organotin Derivatives.

The physicochemical properties of the synthesized organotin metal complexes are described below

**Sn (Sulphanilamide)**

White powder, m. p. 165°C, FT-IR (cm⁻¹) 3476s, 3372s, 3264s ν(NH), 1593 ν (CH=CH), 1143 ν(C–N), 1303 ν(S=O) ¹H NMR (DMSO-D₆, ppm), 7.43-7.46d; 6.56-6.0d (−C₆H₄−), 6.9s (−NH₂), 5.81s(H₃N-SO₂), ¹³C NMR (DMSO-D₆, ppm), 152.38 (C-1), 112.88 (C-2/6), 127.88 (C-3/5), 130.45 (C-4).

**Sn¹ (Triphenylin derivative of sulphanilamide) [(Ph₃Sn)-sulphanilamide]**

Yield (81%), m. p. 150 °C, Elemental Analysis: calculated for C₃₆H₃₆N₂O₅Sn: C, 75.15; H, 6.70; N, 4.68; FT-IR (cm⁻¹) 3373s ν(NH), 3067 ν(CH), 1594 ν (CH=CH), 1145 ν(C–N), 1304 ν(S=O), 438 ν(Sn–N), ¹H NMR (DMSO-D₆, ppm), 7.83-7.84d; 6.57-6.59d (−C₆H₄−), 7.41-7.45m; (−C₆H₄−), 6.89s (−NH₂), 5.81s (−NH₂) ¹³C NMR (DMSO-D₆, ppm), 151.89 (C-1), 112.38 (C-2/6), 128.37 (C-3/5), 129.0 (C-4), 129.97 (C-7), 136.19 (C-8/12), 128.03 (C-9/11), 127.38 (C-10).

**Sn² (Diphenyltin derivative of sulphanilamide) [(Ph₂Sn)-sulphanilamide]²**

Yield (73%), semisolid, Elemental Analysis: calculated for C₃₆H₃₇N₂O₃Sn: C, 74.89; H, 3.64; N, 6.32; found: C, 48.68; H, 3.71; N, 6.22; FT-IR (cm⁻¹) 3369s ν(NH), 3069 ν(CH), 1595 ν (CH=CH), 1147 ν(C=N), 1310 ν(S=O), 452 ν(Sn–N), ¹H NMR (DMSO-D₆, ppm), 7.91-7.95d; 6.55-6.57d (−C₆H₄−), 6.88s (−NH₂), 6.80s (−NH–SO₂), 7.42m, 7.44m; (−C₆H₄−), ¹³C NMR (DMSO-D₆, ppm), 151.9 (C-1), 112.37 (C-2/6), 134.75 (C-3/5), 129.94 (C-4), 127.06 (C-7), 136.33 (C-8/12), 127.75 (C-9/11), 127.38 (C-10).

**Sn³ (Tributyltin derivative of sulphanilamide) [(Bu₃Sn)-sulphanilamide]³**

Yield (65%), m. p. 180°C, Elemental Analysis: calculated for C₃₆H₃₈N₂O₃Sn: C, 48.02; H, 8.06; N, 3.73; found: C, 47.95; H, 8.15; N, 3.61; FT-IR (cm⁻¹) 3372s ν(NH), 2976 ν(CH), 1594 ν (CH=CH), 1144 ν(C–N), 1304 ν(S=O), 477 ν(Sn–N), ¹H NMR (DMSO-D₆, ppm), 7.45-7.47d; 6.58-6.60d; (−C₆H₄−), 6.90s (−NH₂), 5.82s (−NH–SO₂), 0.90d (−CH₃), 1.31-1.63m; (−CH₃–CH₂−), 1.29m; (−Sn–CH₂−)¹³C NMR (DMSO-D₆, ppm), 151.88 (C-1), 112.37 (C-2/6), 129.96 (C-3/5), 127.38 (C-4), 13.62 (C-7), 26.22 (C-8), 27.71 (C-9), 21.02 (C-10).

**Sn⁴ (Dibutyltin derivative of sulphanilamide) [(Bu₂Sn)-sulphanilamide]⁴**

Yield (59%), semisolid, Elemental Analysis: calculated for C₃₆H₃₇N₂O₃Sn: C, 41.71; H, 6.00; N, 6.95; found: C, 41.85; H, 5.87; N, 7.11; FT-IR (cm⁻¹) 3372s ν(NH), 2975 ν(CH), 1596 ν (CH=CH), 1148 ν(C–N), 1338 ν(S=O), 411 ν(Sn–N), ¹H NMR (DMSO-D₆, ppm), 7.42-7.47d; 6.56-6.11d; (−C₆H₄−), 6.88s; (−NH₂), 5.80s; (−NH–SO₂), 0.87-0.88m; (−CH₃), 1.65m; (−CH₃–CH₂−) 1.16-1.29m; (−CH₃–), 13C NMR (DMSO-D₆, ppm), 152.02 (C-1), 112.35 (C-2/6), 129.94 (C-3/5), 127.36 (C-4), 8.68 (C-7), 27.31 (C-8), 25.65 (C-9), 13.48 (C-10).

**Sn⁵ (Trimethyltin derivative of sulphanilamide) [(Me₃Sn)-sulphanilamide]⁵**

Yield (67%), m. p. 155°C, Elemental Analysis: calculated for C₃₆H₃₇N₂O₃Sn: C, 78.95; H, 4.86; N, 5.63; found: C, 79.09; H, 5.00; N, 5.42; FT-IR (cm⁻¹) 3373s ν(NH), 2958 ν(CH), 1595 ν (CH=CH), 1146 ν(C–N), 1311 ν(S=O), 411 ν(Sn–N), ¹H NMR (DMSO-D₆, ppm), 7.45-7.48d; 6.59-6.61d; (−C₆H₄−), 6.92s; (−NH₂), 5.83s; (−NH–SO₂), 0.87s (−CH₃), ¹³C NMR (DMSO-D₆, ppm), 151.88 (C-1), 112.4 (C-2/6), 129.96 (C-3/5), 127.39 (C-4), 5.2 (C-7).

**Sn⁶ (Dimethyltin derivative of sulphanilamide) [(Me₂Sn)-sulphanilamide]⁶**

Yield (55%), m. p. 140°C, Elemental Analysis: calculated for C₃₆H₃₇N₂O₃Sn: C, 30.12; H, 3.79; N, 8.78; found: C, 29.98; H, 3.68; N, 8.87; FT-IR (cm⁻¹) 3344s
v(NH) 3066, 2984 v(CH), 1593 v(CH=CH), 1144 v(C=N), 1335 v(S=O), 447 v(Sn–N). 1H NMR (DMSO-d6, ppm), 7.44–7.46d; 6.58–6.60d; (−CH3), 6.9s (−NH−), 5.83s (−NH–SO2), 1.17s (CH3–). 13C NMR (DMSO-d6, ppm), 151.89 (C–1), 112.38 (C–2/6), 129.93 (C–3/5), 127.37 (C–4), 8.69 (C–7).

Antibacterial activity
The antibacterial activity of organotin derivatives of sulphanilamide was tested against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) using the agar well diffusion method. Cefixime and DMSO were used as positive and negative controls respectively. The plates were incubated at least 24 mm apart. The plates were incubated immediately at 37 °C for 20 h. The activity was determined by measuring the diameter of zones showing complete inhibition in millimetres. Growth inhibition was calculated with reference to positive control.

Antifungal activity
The antifungal activity of synthesized organotin derivatives of sulphanilamide were tested against *Aspergillus Flavus*, *Aspergillus niger*, *Rhizoctonia Solani*, *Aspergillus Fumigatus* and *Mucor* by using the tube diffusion test. Terbinafine (200 mg/ml) was used as standard drug, positive control and DMSO as negative control. The amount of growth inhibition was calculated as:

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

A = Diameter of fungal colony in control plate
B = Diameter of fungal colony in test plate

RESULTS AND DISCUSSION
The organotin derivatives of sulphanilamide were mostly solid except diphenyltin and dibutyltin these were semisolid and all derivatives were physically stable. FT-IR spectra range 4000-400cm⁻¹ were measure and the three sharps peaks of NH₂, and NH₂–SO₂ between the range of 3350 and 3250cm⁻¹ (stretching) become single peak around 3300-3400cm⁻¹ symmetric. The peak of CH aliphatic 2850-2960cm⁻¹ (stretching sp³) and CH aromatic at 3000cm⁻¹ (stretching sp²) appeared and were more profound in butyltin, methyltin and phenyltin derivatives respectively. 1HNMR and 13CNMR shows the no. of proton and carbon according to structure in the expected ranges. Elemental analysis also corresponds well with the calculated values. Antibacterial and antifungal activities were done and the results showed that sulphanilamide were inactive against the microbes but interestingly the synthesized organotin derivatives were active to some extent. The highest activity was exhibited by Sn³ (Tributyltin derivative of sulphanilamide) [(BuSn)₃-sulphanilamide] against the pathogenic bacterial as well as fungal strains. The results of the biological screening are given below in table 1 and figure 1 and figure 2 graphical illustrations of the results are shown.

Table 1: Antibacterial activity of Organotin Derivatives of Sulphanilamide

<table>
<thead>
<tr>
<th>Samples</th>
<th><em>Staph. aureus</em></th>
<th><em>E.coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphanilamide</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sn¹</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Sn²</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Sn³</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Sn⁴</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Sn⁵</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Sn⁶</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Cefixime</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Antifungal activity of the organotin derivatives of Sulphanilamide

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>A. Flavus</em></th>
<th><em>A. Niger</em></th>
<th><em>A. Solani</em></th>
<th><em>A. Fumigatus</em></th>
<th><em>Mucor</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulphanilamide</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Sn¹</td>
<td>11</td>
<td>14</td>
<td>15</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Sn²</td>
<td>11</td>
<td>14</td>
<td>15</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Sn³</td>
<td>26</td>
<td>25</td>
<td>14</td>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>Sn⁴</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sn⁵</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sn⁶</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Terbinafine</td>
<td>28</td>
<td>33</td>
<td>35</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 2: Graph representing Antibacterial activity of Organotin Derivatives of Sulphanilamide

Figure 3: Graph Representing Antifungal activity of the organotin derivatives of Sulphanilamide.
CONCLUSION

Organotin derivatives of sulphanilamide were synthesized in appreciable yield and characterized spectroscopically. Newly synthesized Organotin derivatives of sulphanilamide were observed to be more active than their parent drug sulphanilamide against bacterial and fungal strains. Hence we conclude that metal complexation enhances the pharmacological potential of the drug.

AKNOWLEDGEMENT

We are thankful to Higher Education Commission of Pakistan, for their financial support and Dr. IhsanUlHaq, lecturer, department of pharmacy, Quaid-e-Azam university Islamabad for assisting in biological screening.

REFERENCE