**In vitro** Antibacterial Activity of Dithiocarbamate Organotin(IV) Complexes towards *Staphylococcus aureus*

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**ABSTRACT**

The complexes [Sn\(\{\text{S}_2\text{CN(CH}_2\text{)}_4\}\_2\text{Cl}_2\}], 1, [Sn\(\{\text{S}_2\text{CN(CH}_2\text{)}_4\}\_2\text{Ph}_2\}] \(\text{Ph=phenyl}\), 2, [Sn\(\{\text{S}_2\text{CN(CH}_2\text{)}_4\}\_2\text{Ph}_3\}], 3, [Sn\(\{\text{S}_2\text{CN(CH}_2\text{)}_4\}\_2\text{n-Bu}_2\}] \(\text{n-Bu=}-\text{butyl}\), 4, [Sn\(\{\text{S}_2\text{CN(CH}_2\text{)}_4\}\text{Cy}_3\}] \(\text{Cy=Cyclohexyl}\), 5, [Sn\(\{\text{S}_2\text{CN(C}_2\text{H}_5\text{)}_2\}\_2\text{Cl}_2\}], 6, [Sn\(\{\text{S}_2\text{CN(C}_2\text{H}_5\text{)}_2\}\_2\text{Ph}_2\}], 7, [Sn\(\{\text{S}_2\text{CN(C}_2\text{H}_5\text{)}_2\}\_2\text{Ph}_3\}], 8, [Sn\(\{\text{S}_2\text{CN(C}_2\text{H}_5\text{)}_2\}\_2\text{Ph}\}], 9 and [Sn\(\{\text{S}_2\text{CN(C}_2\text{H}_5\text{)}_2\}\_2\text{Cy}_3\}], 10 were used in this work. The **in vitro** antibacterial activity study was performed against *Staphylococcus aureus* (ATCC 25923) and all complexes were active. The activity was measured in terms of inhibition zones (mm) and minimum inhibitory concentration test, MIC (\(\mu\)g mL\(^{-1}\)). In terms of inhibition zones compounds 6 and 9 presented the best results. Complexes where the –SnPh\(_3\) moiety is present possess the smaller MIC values. The apparent contradiction of the tests might be related to diffusion properties of the organotin complexes.

**Keywords:** Tin(IV) complexes; dithiocarbamate ligands; *Staphylococcus aureus*.

**1. INTRODUCTION**

*Staphylococcus aureus* is known as a versatile pathogenic bacterium which causes a wide variety of community and hospital-acquired infections usually associated with antimicrobial resistance. It is frequently involved in cases of resistant infections in immuno-compromised patients. Methicillin-resistant *S. aureus* (MRSA) has been responsible for a number of morbidity cases and even deaths in healthy children and adults...
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III. This bacterium has recently acquired resistance to virtually all antimicrobials /2/ and has overcome all the therapeutic agents that have been developed in the last 50 years. The clinical importance of S. aureus infections has increased in the last years due to the high number of hospital infections related to multi-resistant isolates /3, 4/. Therefore, the search for a novel antimicrobial is urgently required.

Several works report interesting biological studies concerning dithiocarbamic acids /5/. Organotin compounds are known for their antifungal, antibacterial and antitumoral activities /6-8/. In the last few years we have been interested in the biological activity of organotin complexes. In a previous work we have structurally authenticated some pyrrolidine dithiocarbamate tin(IV) complexes and studied its antifungal activity against Candida albicans, in comparison to the commercially available nystatin /9/. In addition, antifungal and cytotoxic activities of some thiosemicarbazone tin(IV) complexes have been screened /10/. Herein we report the outcome of the in vitro antibacterial activity studies of two series of tin(IV) dithiocarbamates complexes against S. aureus.

2. EXPERIMENTAL

The complexes 1-5 have been prepared by us and published elsewhere /9/. Compounds 6-9 were synthesised according to literature procedure /11/. Finally, the new compound [Sn(S₂CN(C₅H₅)₂)Cy₃] 10 was prepared in a similar manner /12/.

Staphylococcus aureus (ATCC 25923) was aerobically grown in either nutrient broth (1% peptone, 0.5 % beef extract, 0.5 % NaCl, pH 6.0) or agar (nutrient broth plus 2% agar) at 37 °C in all tests. The microorganism suspension used in the experiments corresponds to 10⁵ - 10⁶ CFU mL⁻¹ (for minimum inhibitory concentration test, MIC) and to 10⁸ CFU mL⁻¹ (for agar disk diffusion test), which has been determined by plate counting. Agar disk diffusion test and MIC determination values were performed according to the National Committee of Clinical Laboratory Standard Guidelines /13/.

For the agar disk diffusion test a sample of 100 μL overnight cultures was added to 25 mL of nutrient agar in sterile Petri dishes. Solutions of the complexes were prepared in dichloromethane in the concentrations of 0.09; 0.18; 0.44; 0.89 and 3.54 mmol L⁻¹ and aliquots of 10 μL were pipetted and dropped onto each disk, resulting in an amount of mass in each of 0.5; 1.0; 2.5; 5.0; 10.0; and 20.0 μg, respectively. All experiments were carried out in triplicate. A control disk containing dichloromethane solvent was included and yielded an inhibition zone of 7 mm.

For the MIC experiment, serial dilutions (1:2) of the compounds were performed on nutrient broth. The activity of the compounds was compared to control, where cells were grown in the presence of dichloromethane, solely.

3. RESULTS AND DISCUSSION

The structures for all compounds displayed in Figure 1 are based on previous synthetic works /9, 11/ and for compound 10 it results from spectroscopic data /12/.
The agar diffusion test was used as initial screening for the biological activity of all compounds and ligands. The inhibitory effect of the solvent (dichloromethane) has shown a constant inhibition zone of 7.0 mm. Thus, inhibition zones larger than this value indicated antibacterial activity of the compounds. The dithiocarbamate salts were not active against the bacterium, Table 1. On the other hand, all complexes have shown remarkable activity at the screened concentrations. The results were plotted against concentration to show how it affects the bacterial growth, Figure 2.

**Table 1**

Inhibition zones (mm) and MIC values for complexes 1-10 and ligands against *S. aureus* (ATCC 25923).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition zone / mm*</th>
<th>MIC / μg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>** 10.2**</td>
<td>14.0</td>
</tr>
<tr>
<td>2</td>
<td>** 9.3**</td>
<td>12.2</td>
</tr>
<tr>
<td>3</td>
<td>10.4</td>
<td>14.1</td>
</tr>
<tr>
<td>4</td>
<td>** 9.0**</td>
<td>11.2</td>
</tr>
<tr>
<td>5</td>
<td>9.0</td>
<td>11.0</td>
</tr>
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Table 1 (continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition zone / mm*</th>
<th>MIC / μg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>11.0 13.0 14.0 15.4 15.4 20.0</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>10.0 11.1 13.1 14.0 14.0 15.0</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>9.5 10.5 12.0 15.0 15.0 15.5</td>
<td>1.0</td>
</tr>
<tr>
<td>9</td>
<td>11.5 12.0 14.5 16.5 16.5 18.0</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>8.0 8.0 11.0 11.5 11.5 12.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Concentration / mmol L⁻¹ (μg mL⁻¹) (50) (100) (250) (500) (1000) (2000)

*Standard error of ±1 mm. ** Values corresponding to inhibition of 7.0 mm.

Compounds containing diethyl ligands were more active than pyrrolidine compounds, Figure 2, since they displayed higher inhibition zones. These experiments revealed that compounds 6 and 9 are the best. For compounds 6 and 9 a tendency is observed of higher activity as the concentration increases. The inhibition zones reach the top values, 20 and 18 mm respectively, at concentration of 3.54 mmol L⁻¹. A similar trend is detected for compound 3, however the inhibition is not as great as that detected for the previous compounds. At low concentrations, the other derivatives also inhibit the development of bacteria colony; however, this effect soon stabilises. The more stable are compounds 2, 4, 5, 8 and 10.

Commercial disks with clinical drugs - tetracycline (30 μg), streptomycin (10 μg) and erythromycin (15 μg) - produce inhibition zones of 24.0-30.0; 14.0-22.0; 22.0-30.0 mm, respectively, towards NCCLS (M100-S14). The compounds 1, 3, 6 - 9 reported here compare closely to streptomycin.

The inhibition zone experiments provided an idea about the antibacterial potential of the complexes, and it was expected that the MIC tests would support the results. However, a positive correlation was not observed between the two independent experiments. Although many authors recommend the use of either test to differentiate sensitive from resistant S. aureus isolates, this correlation is not always observed for different compounds /14/. It is known that the antibacterial action of drugs differs from solution to solid media. So, the apparent paradox detected in the present work possibly relates to differences of behaviour of tin compounds when in solution or in pseudo solid medium (agar). It is possible that the mobility of the complexes in agar is limited by weak interactions of them with this pseudo-solid medium. Therefore, they do not diffuse as well as other drugs through agar; thus, higher concentrations are required for effective testing. The MIC values of the compounds for the S. aureus ATCC 25923 varied from 1 to 70 μg mL⁻¹, Table 1. In this test, as expected, the triphenyl derivatives, compounds 3 and 8, exhibited the smallest MIC values, followed by compounds 4, 5 and 10. It has been previously reported that organotin compounds containing three R-Sn bonds (R corresponding to organic group) present smaller toxicity as well as higher antimicrobial activity compared to mono and bi-substituted complexes. Moreover, -SnR₃ containing complexes (R = Bu, Cyclohexyl and Ph), in this order, present higher antifungal activity compared to other alkyl complexes /15/. Compounds 10 and 4 also present moderate activity. The latter is probably due to the presence of non-coordinate sulphur atoms. The two free S atoms may serve as electron-rich centres for extra bonding at the membrane, essential for the survival of the bacterium cell.
Fig. 2: Inhibition zone versus concentration for complexes: (I) pyrrolidinedithiocarbamate derivatives (1)-(5) and (II) diethyldithiocarbamate derivatives (6)-(10) against *S. aureus* (ATCC 25923).
4. CONCLUSIONS

The results presented in this study show that dithiocarbamates tin(IV) complexes, in addition to their potential as antifungal compounds, are promising candidates as antibacterial drugs. In terms of inhibition zones, the diethyldithiocarbamates tin(IV) complexes are more active than the pyrrolidine analogues. Compounds 6 and 9 exhibit a pronounced antibacterial effectiveness. On the other hand, in terms of MIC the more active are those which contained SnR₃ fragment {R = Ph and Cy}. Studies of toxicity are still required.

5. ACKNOWLEDGEMENTS

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6. REFERENCES


12. Complex 10 was isolated as white solid soluble in polar organic solvents. Yield (1.55g, 81 %). M.p. = 132 °C. Elemental analysis: Found: C, 53.11; H, 8.01; N, 2.55 %. Calc. for C_{23}H_{43}SnNS_2: C, 53.52; H, 8.34; N, 2.71 %. IR (ν cm^{-1}, KBr): 262w and 292m (Sn-C); 958m (C-S); 1498s (C-N + C=N). ^1H NMR (CDCl_3): δ 1.29 (t, 2CH_2CH_3); 3.92 (m, 2NCH_2); 1.48-2.02 (m, 3C_6H_11). ^13C{^1H} NMR (CDCl_3): δ 12.23 (2CH_2CH_3); 26.77 (1J(13C-119Sn) 8 Hz); 29.36 (2J(13C-119Sn) 72 Hz); 32.05 (2J(13C-119Sn) 18 Hz); 34.71 (1J(13C-119Sn) 263 Hz); 49.63 (2CH_2CH_3); 198.57 (SCN). ^119Sn{^1H} NMR (CHCl_3): δ-170. ^119Sn Mössbauer spectroscopy: IS=1.32 mms^{-1} and QS=1.78 mms^{-1}.


