SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF 2, 2′-DISULFANEDIYLDIBENZAMIDE
S Jhaumeer Laulloo*, D Sreekisson and Minu G Bhowon*

Department of Chemistry, Faculty of Science, University of Mauritius, Reduit

E-mail: sabina@uom.ac.mu

Abstract
Diaryl disulfides compounds are important class of compounds having multifaceted applications in pharmaceutical industry as well as in the field of material science. Three dialkyl 2,2′-disulfanediyl dibenzamides of enhanced lipophilic character were synthesized by introducing long alkyl chains C8-C12 at the amino end. These benzamides were characterized by elemental, IR, NMR and 2D NMR. These compounds showed interesting antibacterial activity over a panel of gram positive and gram negative bacteria. The compound containing the C12-alkyl chain was found to be more active.

Keywords: diaryl disulfides, dithiosalicylic acid, diphenyldisulfides, anti-bacterial.

1. Introduction
Organosulfur compounds are indispensable chemical substances to life and widely exist as biological macromolecules [1], materials [2], pharmaceuticals, agricultural and chemical agents [3]. Aromatic sulfides represent a privileged class of organosulfur compounds which exhibit broad applications in various research fields [4-9]. A few natural-occurring disulfides derived from garlic including ajoene, allicin, vinyl dithiin and S-allyl mercaptocysteine have exhibited important anti-microbial, anti-fungal and anti-cancer properties [8]. Moreover, many active compounds such as proteins, prodrugs, and vulcanizing agents also have disulfide linkages.

A number of diaryl disulphide compounds have been reported to exhibit, anti-oxidant, anti-HIV and anti-bacterial properties [9, 10] and also as novel stabilizers of tumor suppressor Pdcd4 [11].
Lipophilicity is a physicochemical property of crucial importance in medicinal chemistry as it affects drugs transport through the lip structure as well as drugs interaction with the target protein. Hence increasing the alkyl chain length enhances the anti-bacterial properties to a critical point [12, 13]. Therefore, in continuation of our efforts in the synthesis of biologically active organo-sulfur compounds, we herein report the synthesis of disulfanediyldibenzamides of enhanced lipophilic character by introducing long alkyl chain C8-C12 in order to deliver electrophilic sulfur species more effectively into the bacterial cell wall.

2. Materials and methods

2.1 Chemicals and instrumentation

Octanoic acid, decanoic acid, dodecanoic acid, bis(aminophenyldisulfide) and dichloromethane (DCM) were brought from Sigma-Aldrich. Thionyl chloride was obtained from Fluka chemica. Tetrahydrofuran (THF) was purchased from SD Fine-Chem Limited (SDFCL). The melting point of the compounds was obtained using a stuart automatic melting point SMP40. Infrared spectra were recorded on a Bruker Alpha FTIR spectrometer (400-4000 cm\(^{-1}\)). \(^{1}\)HNMR, \(^{13}\)CNMR, DEPT, 2D NMR spectra were obtained using a Bruker Spectra spin NMR spectrometer using CDCl\(_3\) as solvent. CHNS percentage was obtained from a Eurovector EA 3000 elemental analyser.

2.2 Synthesis

The dialkyl 2, 2’-disulfanediyldibenzamide (1-3) were synthesized by the reaction of bis-amino phenyl disulphide with fatty acid chloride. The fatty acid chlorides were synthesized from their corresponding fatty acid (octanoic, decanoic, and dodecanoic acid) by refluxing with SOCl\(_2\) (2ml) in DCM (15ml). Simple distillation and rotary evaporator were used to remove the solvents (DCM and thionyl chloride). The acyl chloride formed was then refluxed with bis-amino phenyl disulphide in THF (40ml). The reaction was quenched with distilled water (50ml) and the solid formed was filtered and recrystallized using THF as solvent.

**Dioctyl 2, 2’-disulfanediyldibenzamide (1):** Yield: 51.1%. M.P: 58.7 °C. FT-IR (V, cm\(^{-1}\)): 2953 (C-H\(_{\text{arom}}\)), 2852, 2922 (C-H\(_{\text{aliphatic}}\)), 464 (S-S), 1654 (CO\(_{\text{amide I}}\)), 1520 (amide II), 1324 (amide III), 3267 (N-H). \(^{1}\)HNMR (250 MHz, CDCl\(_3\), \(\delta\), ppm): 7.39 (d, H\(_{\text{arom}}\), J=8Hz), 7.36 (t, H\(_{\text{arom}}\), J=8Hz), 7.00 (t, H\(_{\text{arom}}\), J=7Hz), 8.41 (d, H\(_{\text{arom}}\), J=8Hz), 2.16 (m, CH\(_2\), J=7Hz), 1.2-2.1 (H\(_{\text{aliph}}\), m,
12H, 6xCH₂), 0.8-0.9 (t, 6H, 2xCH₃), 8.4 (NH). ¹³CNMR (62.9MHz, CDCl₃, δ, ppm): 14.1, 22.6, 25.4, 29.0, 29.2, 31.7, 37.8, 120.9, 123.4, 124.2, 132.2, 136.5, 139.9, 171.5 (CO), Anal. Calcd. For C₂₈H₄₀S₂N₂O₂ (500.76): C, 67.20%; H, 8.00%; N, 5.60%; S, 12.8%. Found: C, 67.2%; H, 8.9%; N, 4.8%; S, 11.1%.

Didecyl 2, 2’-disulfanediyl dibenzamide (2): Yield: 29%. M.P: 78.9 ⁰C. FT-IR (V, cm⁻¹): 2849, 2917 (C-H aliphatic), 2952 (C-H aromatic). ¹HNMR (250 MHz, CDCl₃, δ, ppm): 7.39 (d, H arom, J=8Hz), 7.36 (t, H arom, J=9Hz), 7.00 (t, H arom, J=7Hz), 2.18 (m, CH₂, J=6Hz), 0.89 (t, 6H, 2xCH₃). ¹³CNMR (62.9MHz, CDCl₃, δ, ppm): 14.1, 22.6, 29.0, 29.2, 29.3, 29.4, 29.5, 31.9, 37.8, 120.9, 123.4, 124.1, 132.2, 136.5, 136.9 (C arom), 171.4 (CO), Anal. Calcd. For C₃₂H₄₈S₂N₂O₂ (556): C, 69.1%; H, 8.6%; N, 5.00%; S, 11.5%. Found: C, 67.7%; H, 8.9%; N, 4.9%; S, 10.9%.

Didodecyl 2, 2’-disulfanediyl dibenzamide (3): Yield: 67%. M.P: 87 ⁰C. FT-IR (V, cm⁻¹): 2849, 2917 (C-H aliphatic), 2952 (C-H aromatic), 1525 (amide II), 1379 (amide III), 3269 (N-H). ¹HNMR (250 MHz, CDCl₃, δ, ppm): 7.39 (d, H arom, J=8Hz), 7.36 (t, H arom, J=9Hz), 7.00 (t, H arom, J=7Hz), 8.40 (d, H arom, J=8Hz), 2.18 (m, CH₂, J=6Hz), 0.89 (t, 6H, 2xCH₃). ¹³CNMR (62.9MHz, CDCl₃, δ, ppm): 14.1, 22.7, 29.4, 29.5, 31.9, 37.9, 120.9, 123.4, 132.2, 136.5, 136.9 (C arom), 171.4 (CO), Anal. Calcd. For C₃₆H₅₆S₂N₂O₂ (612): C, 70.5%; H, 9.2%; N, 4.6%; S, 10.5%. Found: C, 70%; H, 9.0%; N, 4.3%; S, 9.8%.

2.3 Anti-bacterial activity

The bioactivity of dialkyl 2, 2’- disulfanediyl dibenzamides (1 & 3) was tested against 3 gram positive strains (Staphylococcus aureus ATTC 25923, Staphylococcus epidermidis ATTC 1228, and Bacillus cereus ATTC 11778) and 2 gram negative strains (Klebsiella pneumonia ATTC 13883, Escherichia coli ATTC 22922) using Broth dilution method [13] and the activity was expressed as the Minimum Inhibitory Concentration (MIC). The wells of the 96-Elisa plate were filled with 50µl bacteria suspension and 50µl of compounds (1 or 3) of varying concentrations and were adjusted to 0.5 McFarland in physiological solution. The microplates were then incubated for 24hr. 20µl Iodonitrotetrazolium was added and incubated for further 30 minutes.
MIC was obtained as the lowest sample concentration that caused the colour change (yellow to pink) and exhibit complete inhibition of bacterial growth. CTAB (Cetyl trimethyl ammonium bromide) was used as positive control and % DMSO as negative control.

3 Results and discussions

3.1 Chemistry

Fatty acids (octanoic acid, decanoic acid, dodecanoic acid) were refluxed with SOCl$_2$ (2ml) in DCM (15ml) to its corresponding acyl chloride. Bis amino phenyl disulphide was added to the acyl chloride using THF (40ml) and base NEt$_3$ (0.5ml) and refluxed to yield the benzamides (1 - 3) with long alkyl chain C8-C12 (scheme 1).

![Chemical structure](image)

Scheme 1: Synthesis of 2, 2'-disulfanediyldibenzamides 1-3.

The disappearance of the NH$_2$ peak at 1610 and appearance of the new band in the region of 1654-1659 cm$^{-1}$ in IR-Spectra of 1-3 indicates the formation of the amide group. The aromatic and aliphatic C-H’s appear in the regions of 2900-3000 and 2800-2900 cm$^{-1}$ respectively. The S-S bond appears at 462-463 cm$^{-1}$. 
In $^1$H NMR spectra of the compounds 1-3, the CH$_3$ protons appear as a triplet at δ 0.8-0.9 ppm. The methylene protons attached to the carbonyl amide appear more downfield at δ 2.17-2.34 ppm while the other methylene protons appear at δ 1.2-2.1 ppm. The aromatic protons appears at δ 7.0-8.4 ppm.

In the $^{13}$CNMR and DEPT of the compounds 1-3, the methyl carbon appears at δ 14.1 ppm. The aromatic carbons appear at δ 120-139 ppm and the peak at δ 171 ppm corresponding to the amide carbon. The more downfield methylene appear at δ 67.1 ppm as it is attach to the amide group and the other methylene carbons appear at δ 22.7-37.8 ppm.

Table 1: Spectral data of compounds 1-3

<table>
<thead>
<tr>
<th>Compd</th>
<th>IR/ cm$^{-1}$</th>
<th>$^1$HNMR (ppm)</th>
<th>$^{13}$CNMR (ppm)</th>
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<tr>
<td></td>
<td>Amide I</td>
<td>Amide II</td>
<td>Amide III</td>
</tr>
<tr>
<td>1</td>
<td>1654</td>
<td>1520</td>
<td>1324</td>
</tr>
<tr>
<td>2</td>
<td>1659</td>
<td>1522</td>
<td>1378</td>
</tr>
<tr>
<td>3</td>
<td>1659</td>
<td>1522</td>
<td>1377</td>
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</table>
In order to assign the chemical shift of the different protons and carbons, the 2D NMR (COSY and HMBC) of the compounds (1-3) were carried out. The COSY and HMBC spectra of the benzamide 3 are given in Figure 1. The complete assignment of the different protons and carbons is summarized in Table 2.

(a)

(b)
Table 2: $^1$H, $^{13}$C NMR, COSY and HMBC correlations for 3.

<table>
<thead>
<tr>
<th>Atom</th>
<th>$^1$HNMR</th>
<th>$^{13}$CNMR</th>
<th>DEPT</th>
<th>COSY</th>
<th>HMBC</th>
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<tr>
<td>1</td>
<td></td>
<td>139.8</td>
<td></td>
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<td>2</td>
<td>7.39 (2H, d, J=8Hz)</td>
<td>120.9</td>
<td>120.9</td>
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<tr>
<td>3</td>
<td>7.33 (2H, t, J=8Hz)</td>
<td>124.2</td>
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<tr>
<td>4</td>
<td>7.00 (2H, t, J=8Hz)</td>
<td>136.4</td>
<td>136.4</td>
<td>3, 5</td>
<td>2, 6</td>
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<tr>
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<td>8.40 (2H, d, J=8Hz)</td>
<td>132.2</td>
<td>132.2</td>
<td>4</td>
<td>1, 3</td>
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<tr>
<td>6</td>
<td>-</td>
<td>123.5</td>
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</tr>
<tr>
<td>7</td>
<td>-</td>
<td>171.6</td>
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<td></td>
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<tr>
<td>8</td>
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<td>1.61 (4H, t, J=7Hz)</td>
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<td>10-17</td>
<td>1.27 (32, m)</td>
<td>37.8, 31.9, 29.6, 29.5, 29.3, 29.2, 25.4, 22.7</td>
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<tr>
<td>18</td>
<td>0.89 (6H, t, J=7Hz)</td>
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<td>N-H</td>
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3.2 Anti-bacterial

The dialkyl 2, 2’-disulfanediyl dibenzamides (1 & 3) were evaluated for their anti-bacterial properties against a panel of Gram positive and Gram-negative strains. Preliminary results showed that compound 3 having a C-12 alkyl chain showed better anti-bacterial properties with the lowest MIC (0.03 mg/ml) against Bacillus Cereus.

References
