European Journal of Chemistry

Journal webpage: www.eurjchem.com

Synthesis, antibacterial activities and phospholipid membrane interactions of novel dialkyl 2,2'-disulfanediyldibenzoates

Sabina Jhaumeer Laulloo [1,*], Minu Gupta Bhowon [1], Matthew Akerman [2], Nausheen Joondan [1] and Marie Jessika Momus [1]

[1] *Department of Chemistry, Faculty of Science, University of Mauritius, Reduit 80837, Mauritius*
[2] *University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg 3209, South Africa*

** Corresponding author at: Department of Chemistry, Faculty of Science, University of Mauritius, Reduit 80837, Mauritius. Tel.: +230.403.7505. Fax: +230.465.6928. E-mail address: sabina@uom.ac.mu (S.J. Laulloo).*

ARTICLE INFORMATION

DOI: 10.5155/eurjchem.8.1.60-65.1540

Received: 30 December 2016
Received in revised form: 25 January 2017
Accepted: 25 January 2017
Published online: 31 March 2017
Printed: 31 March 2017

KEYWORDS

Lipophilicity
Dithiosalicylic acid
Dithio-bis-benzoates
Antibacterial activity
Phospholipid binding
X-ray crystallography

ABSTRACT

A series of dialkyl 2,2'-disulfanediyldibenzoates with alkyl chain length C_8 to C_{12} were synthesized and characterized by spectral studies. The structure of compound 3 was also confirmed by X-ray crystallography. The compounds were found to possess good antibacterial activity, especially with respect to Gram positive bacteria, and the activity was found to increase with concomitant increase in chain length. Binding studies of compound 3 with the synthetic phospholipid 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) showed that the compound interacts with biological membrane mainly via hydrophobic interactions.

Cite this: *Eur. J. Chem.* **2017**, *8(1)*, 60-65

1. Introduction

Sulfur containing compounds have been a mainstay of organic synthesis as they have broad significance in organic, pharmaceutical and medicinal chemistry [1]. Moreover, many biologically active compounds including proteins, prodrugs and vulcanizing agents have disulfide linkages [2]. A number of diaryldisulfides compounds containing different functional groups have been reported to exhibit various properties such as anti-leishmanial [3], anti-oxidant [4], anti-HIV [5,6] and anti-bacterial properties [7-10].

Lipophilicity is also known to influence the biological activity of various compounds [11,12]. Turos *et al*. reported that increasing alkyl chain length from methyl to butyl in alkyl-aryl disulfide system enhances the anti-bacterial properties of these compounds [13]. Therefore, in continuation of our efforts in the synthesis of biologically active organo-sulfur compounds, we thought of increasing the lipophilic character of 2,2'-dithiodisalicylic acid by introducing long alkyl chain at the carboxylic end in order to deliver electrophilic sulfur species more effectively into the bacterial cell wall. We, herein report the synthesis, critical micelle concentration (CMC) and anti-bacterial properties of new dialkyl 2,2'-disulfanediyldibenzoates containing long alkyl chains. The binding property of the compound **3** with the phospholipid 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) is also investigated.

2. Experimental

2.1. Materials

Dithiosalicylic acid, octanol, decanol, dodecanol, tris buffered saline (0.05 M, pH = 7.4), 1,6-diphenyl-1,3,5-hexatriene (DPH), 0.65 mM phosphorus standard solution, ammonium molybdate (VI) tetrahydrate and hydrogen peroxide (30%) were purchased from Sigma-Aldrich (Germany, UK). Thionyl chloride was bought from Fluka Chemika. Mueller Hinton broth was obtained from Oxoid Ltd (UK). The different bacterial strains were obtained from Microbiologics® (St Cloud, MN, USA). Cetyl trimethyl ammonium bromide (CTAB) was obtained from BDH Laboratory Supplies (UK). L-Ascorbic acid was obtained from S.D. fine chemicals (India). The synthetic 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was obtained from Avanti Polarlipids, Inc., USA.

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.8.1.60-65.1540

2.2. Instrumentation

Melting points were determined using a Stuart automatic melting point SMP40 and were uncorrected. Infrared spectra were recorded on a Bruker Alpha FT-IR spectrometer in the range 400-4000 cm^{-1} on a diamond cell. ^1H and ^{13}C NMR spectra were recorded at 250 and 62.9 MHz, respectively, on a Bruker Spectro Spin NMR spectrometer using $CDCl_3$. Carbon, hydrogen and sulfur contents were obtained from a Eurovector EA 3000 elemental analyzer. The purity of the synthesized compounds was checked by thin layer chromatography (TLC). Fluorescence intensities were recorded on a LS 55 Perkin Elmer fluorescence spectrophotometer.

The X-ray diffraction data were recorded on a Bruker Apex Duo equipped with an Oxford Instruments Cryojet operating at 100(2) K and an Incoatec micro source operating at 30 W power [14]. Crystal and structure refinement data are given in Table 1. The data were collected with MoKα (λ = 0.71073 Å) radiation at a crystal-to-detector distance of 50 mm. The following conditions were used for the data collection: omega and phi scans with exposures taken at 30 W X-ray power and 0.50° frame widths using APEX2 [15]. The data were reduced with the programme SAINT [15] using outlier rejection, scan speed scaling, as well as standard Lorentz and polarization correction factors. A SADABS semi-empirical multi-scan absorption correction [15] was applied to the data. Direct methods, SHELX-2016 [16] and WinGX [17] were used to solve the structure. All non-hydrogen atoms were located in the difference density map and refined anisotropically with SHELX-2016 [16]. All C-bonded hydrogen atoms were included as idealized contributors in the least squares process.

Table 1. Crystal data and structure refinement details for compound **3**.

Crystal data	Compound 3
Chemical formula	$C_{38}H_{58}O_4S_2$
Molar mass (g/mol)	642.96
Crystal system, space group	Triclinic, *P*-1
Temperature (K)	100(2)
a (Å)	10.8692(7)
b (Å)	11.2046(8)
c (Å)	15.5882(12)
α (°)	84.981(4)
β (°)	81.007(3)
γ (°)	72.699(3)
V (Å^3)	1788.5(2)
Z	2
Radiation type	MoKα
μ (mm^{-1})	0.19
Crystal size (mm)	0.38 × 0.15 × 0.09
Data collection	
Diffractometer	Bruker Apex Duo CCD diffractometer
Absorption correction	Multi-scan, SADABS, Bruker 2012
T_{min}, T_{max}	0.693, 0.746
No. of Measured, independent and observed [$I > 2\sigma(I)$] reflections	23951, 8845, 7106
R_{int}	0.027
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.040, 0.108, 1.05
No. of reflections	8845
No. of parameters	399
No. of restraints	0
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.37, -0.23

2.3. Synthesis

The dialkyl 2,2'-disulfanediyldibenzoates (**1**-**3**) were synthesized by the reaction of the 2,2'-dithio-*bis*-benzoyl chloride with selected fatty alcohols (octanol, decanol and dodecanol). The fatty alcohol (2 eq.) was added to a solution of dithio-*bis*-benzoyl chloride (0.005 moles) which was prepared in-situ in toluene (50 mL) and the resulting solution was refluxed for 24 h. On evaporating the excess solvent, a brown solid was obtained which was recrystallized with ethanol to give the ester as light brown crystals.

Dioctyl 2,2'-disulfanediyldibenzoate (**1**): Yield: (1.58 g) 60%. M.p.: 52-54 °C. FT-IR (KBr, ν, cm^{-1}): 3049, 3066 (C-$H_{arom.}$), 2962, 2950, 2850 (C-$H_{aliph.}$), 1693 (C=O_{ester}), 489 (S-S). ^1H NMR (250 MHz, $CDCl_3$, δ, ppm): 0.88 (t, 6H, *J* = 6.7 Hz, 2CH_3,), 1.27-1.42 (m, 16H, 8CH_2), 1.45 (quintet, 4H, *J* = 6.7 Hz, 2CH_2,),1.79 (quintet, 4H, *J* = 6.7 Hz, 2CH_2,), 4.36 (t, 4H, *J* = 6.7 Hz, 2CH_2,), 7.21 (td, 2H, Ar*H*, *J* = 7.5, 1.1 Hz), 7.41 (td, 2H, Ar*H*, *J* = 7.3, 1.5 Hz), 7.75 (dd, 2H, Ar*H*, *J* = 8.1, 0.8 Hz), 8.04 (dd, 2H, Ar*H*, *J* = 7.7, 1.4 Hz). ^{13}C NMR (62.9 MHz, $CDCl_3$, δ, ppm): 14.1 (CH_3), 22.7, 26.1, 28.1, 28.7, 29.2, 31.8 (6CH_2), 65.7 (O-CH_2), 125.4, 125.9, 131.4, 133.0 (aromatic CH), 127.7, 140.3 (*Tert* aromatic C), 166.6 (C=O). Anal. calcd. for $C_{30}H_{42}O_4S_2$: C, 67.89; H, 7.98; S, 12.08. Found: C, 68.07; H, 8.24; S, 12.54 %.

Didecyl 2,2'-disulfanediyldibenzoate (**2**): Yield: (0.58 g) 20%. M.p.: 55-57 °C. FT-IR (KBr, ν, cm^{-1}): 3065 ($CH_{arom.}$), 2950, 2919, 2850 ($CH_{aliph.}$), 1695 (C=O_{ester}), 489 (S-S). ^1H NMR (250 MHz, $CDCl_3$, δ, ppm): 0.87 (t, 6H, *J* = 6Hz, 2CH_3), 1.25-1.30 (m, 24H, 12CH_2), 1.45 (m, 4H, 2CH_2), 1.79 (quintet, 4H, *J* = 6.7 Hz, 2CH_2), 4.36 (t, 4H, *J* = 6.7 Hz, 2CH_2,), 7.21 (td, 2H, *J* = 7.7, 1 Hz, Ar*H*), 7.41 (td, 2H, *J* = 7.7 Hz, Ar*H*), 7.75 (dd, 2H, *J* = 8.1 Hz, Ar*H*), 8.05 (dd, 2H, *J* = 7.7 Hz, Ar*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$, δ, ppm): 14.1 (CH_3), 22.7, 26.1, 28.7, 28.8, 29.2, 29.5, 30.4, 31.9 (8CH_2), 65.7 (O-CH_2), 125.4, 125.9, 131.4, 133.0 (Arom. CH), 127.7, 140.3 (*Tert* aromatic C), 166.6 (C=O). Anal. calcd. for $C_{34}H_{50}O_4S_2$: C, 69.58; H, 8.59; S 10.93. Found: C, 69.35; H, 8.69; S, 10.92 %.

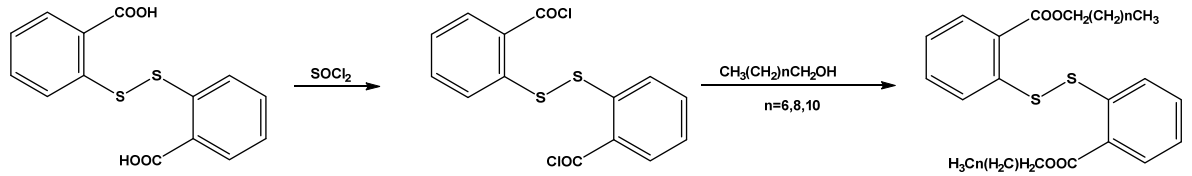
Didodecyl 2,2'-disulfanediyldibenzoate (**3**): Yield: (1.39 g) 45%. M.p.: 167-169 °C. FT-IR (KBr, ν, cm^{-1}): 3052, 3062 ($CH_{arom.}$), 2955, 2919, 2850 ($CH_{aliph.}$), 1701 (C=O_{ester}), 496 (S-S). ^1H NMR (250 MHz, $CDCl_3$, δ, ppm): 0.86 (t, 6H, *J* = 6.6 Hz 2CH_3,), 1.24-1.44 (m, 32H, 16CH_2), 1.45 (m, 4H, 2CH_2), 1.79 (m, 4H, 2CH_2), 4.36 (t, 4H, *J* = 6.6 Hz, 2CH_2), 7.21 (t, 2H, *J* = 7.0, Ar*H*), 7.41 (t, 2H, *J* = 7.0, Ar*H*), 7.75 (d, 2H, *J* = 7.0 Hz, Ar*H*), 8.05 (dd, 2H, *J* = 7.0 Hz, Ar*H*). ^{13}C NMR (62.9 MHz, $CDCl_3$, δ, ppm): 14.1 (CH_3), 22.7, 26.1, 28.7, 28.8, 29.3, 29.4, 29.5, 29.6, 31.9, 32.8 (10CH_2), 65.7 (O-CH_2), 125.4, 125.9, 131.4, 133.0 (Arom. CH), 127.7, 140.3 (*Tert* aromatic C), 166.6 (C=O_{ester}). Anal. calcd. for $C_{38}H_{58}O_4S_2$: C, 70.98; H, 9.09. Found: C, 71.30; H, 9.16 %.

2.4. Antibacterial activity

The antibacterial activities were determined against three Gram positive strains, namely *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228) and *Bacillus cereus* (ATCC 11778, ATCC 10876) and two Gram negative strains, namely *Klebsiella pneumoniae* (ATCC 13883), *Escherichia coli* (ATCC 22922) using the broth dilution method [18]. The antibacterial activity was expressed as the Minimum Inhibitory Concentration (MIC) which was defined as the lowest concentration that inhibits the growth of bacteria. CTAB was used as positive control. All wells were inoculated with 50 μL of a bacterial suspension adjusted to 0.5 McFarland in physiological solution. Microplates were covered and incubated for 24 hr at 37 °C. The minimum inhibitory concentration (MIC) of the surfactants were detected following addition of 20 μL iodonitrotetrazolium chloride (0.4 mg/mL) and incubation at 37 °C for 30 min. Viable microorganisms reduced the yellow dye to a pink color. MIC was defined as the lowest sample concentration that prevented this change and exhibited complete inhibition of bacterial growth.

2.5. Critical micelle concentration

Pyrene was used as a fluorescence probe to determine the CMC of the compounds **1**-**3** in aqueous solution at 25 °C. Stock solution of pyrene in methanol (10 μL, 0.1 mM) was transferred into vials.



Scheme 1

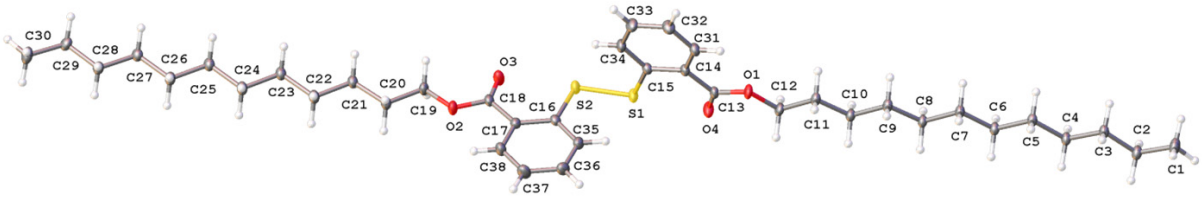


Figure 1. Labelled thermal displacement plot of compound **3** (50% probability surfaces) showing the atom numbering scheme. The hydrogen atoms have been rendered as spheres of arbitrary radius.

After evaporating the methanol, surfactant solutions (3 mL) of varying concentrations were added to the vials to give a final concentration of 1.6 μM of pyrene in each vial. Fluorescence spectra of pyrene were recorded over the spectral range 350-450 nm. The excitation wavelength was kept at 334 nm and the emission was recorded at 373 (I_1) and 384 (I_3) nm. The ratio of the intensities of the first and third vibronic peaks in the fluorescence spectrum of pyrene (I_1/I_3) was recorded as a function of the dithio-*bis*-aryl diesters concentrations to determine the CMCs.

2.6. Phospholipid binding

The hydrophobic interaction of compound **3** with DPPC was determined using DPH as probe using a similar protocol to that reported by our group [19]. Mixtures containing DPPC (2×10^{-5} M), and DPH (5×10^{-8} M) were titrated against varying concentrations of compound **3**, and the quenching of the fluorescence were recorded as a function of compound **3** concentrations. The fraction of bound DPH in the solution was taken to be the ratio of the fluorescence intensity obtained after each μL addition of compound **3** with that of the solution containing a higher concentration of DPPC (1×10^{-3} M) and DPH (5×10^{-8} M). The binding of the dithio-*bis*-aryl diester to DPPC were determined using Equation 1.

$$\frac{(n'-\nu_1)\nu_1}{(n'-\nu^*-\nu_1)[D]} = n'_1 K_1 - \nu_1 K_1 \quad (1)$$

where ν^* is the ratio of bound-probe per total lipid concentration in the presence of the dithio-*bis*-aryl diester; ν_1 is the ratio of bound dialkyl 2,2'-disulfanediyldibenzoates per total lipid concentration; n' is the binding capacity of DPH to DPPC; n'_1 is the maximum value of ν_1 which indicates the probe binding sites which may be replaced by dithio-*bis*-aryl diester; [D] is the free dialkyl 2,2'-disulfanediyldibenzoates concentration; K_1 is the binding constant for the dialkyl 2,2'-disulfanediyldibenzoates-lipid interaction; ν_1 was obtained from Equation 2, using n' and K values (0.0167 and 3×10^6 M^{-1}, respectively) from the binding between DPH to DPPC obtained in previous reports [19]

$$\nu_1 = n'-\nu^*-\frac{\nu^*}{[P]^* K} \quad (2)$$

[P]* represents the free concentration of the probe in the presence of the dialkyl 2,2'-disulfanediyldibenzoates. [D] was taken to be the difference between the total concentration of the dithio-*bis*-aryl diester and the product of ν_1 and total lipid concentration.

3. Results and discussion

3.1. Chemistry

A series of dialkyl 2,2'-disulfanediyldibenzoates were synthesized by the condensation of 2,2'-dithio-*bis*-benzoyl chloride with fatty alcohols of varying alkyl chain lengths (C_8, C_{10} and C_{12}) in a molar ratio 1:2 to yield the corresponding esters as light brown solids in 45-60% yield (Scheme 1).

The presence of the new peak at 1693-1701 cm^{-1} in the IR spectra of compound **1**-**3**, confirms the formation of the ester. The aromatic and aliphatic ν_{CH} in the dithiodibenzoate esters appear at 3049-3066 and 2962-2850 cm^{-1}, respectively. The S-S bond appears at 489-496 cm^{-1}.

In the ^{1}H NMR of compound **1**-**3**, the methyl protons (CH_3) appears as a triplet at δ 0.86-0.88 ppm and the methylene attached to the ester appeared downfield as a triplet at δ 4.36 ppm. The other methylene protons appeared in the region δ 1.24 to 1.79 ppm. The aromatic protons appear at δ 7.21-8.05 ppm.

From the ^{13}C NMR and DEPT spectra of compound **1**-**3**, the tertiary aromatic carbons appear at δ 127.7 and 140.3 ppm while the peak with the highest chemical shift, δ 167 ppm is due to the carbonyl ester. The methyl carbon resonates at δ 14.1 ppm and the downfield peak at δ 65.7 ppm is due to the methylene carbon attached to the ester group. In the aromatic segment the aromatic CH's appear at δ 125.4, 225.9, 131.4 and 133.0 ppm. The methylene protons which appear in the region δ 22.7-32.8 ppm in the ^{13}C NMR and DEPT spectra corroborate with the structures proposed.

3.2. X-ray crystal data of compound 3

Compound **3** crystallized in the triclinic space group *P*-1 with a single molecule in the asymmetric unit and two independent molecules in the unit cell ($Z = 2$). The structure of the asymmetric unit is shown in Figure 1. The two alkyl chains of the molecule are co-linear, and are approximately transverse the *bc* plane.

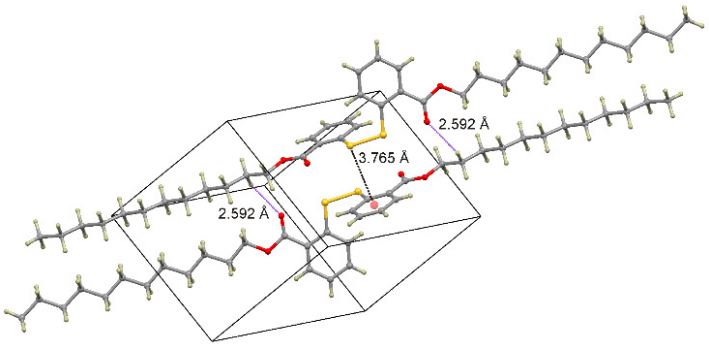


Figure 2. Inversion dimer of compound **3** supported by C–H···O interactions (shown as dashed purple lines) and S···π interactions shown as dashed black lines. The interaction distances are indicated on the diagram. The symmetry code for the interaction dimer is: 1-*x*, 2-*y*, 1-*z*.

In contrast, the phenyl rings are in a near perpendicular orientation with the two six-atom mean planes of the phenyl rings subtending an angle of *ca*. 82°. Despite the long alkyl chain, there is no positional disorder and the structure could be treated with routine refinement methods.

Table 2 shows selected bond lengths and bond angles of Compound **3**. A Mogul structural search [20] shows that the S–S bond, which measures 2.0569(5) Å, is comparable to related bonds reported in the Cambridge Structural Database (CSD) [20]. The mean carbonyl bond length measures 1.205(3) Å, this coupled with the mean O_{ester}–C–$O_{carbonyl}$ bond angle which measures 123.7(2)° highlights the sp^2 hybridized nature of the carbonyl carbon.

Table 2. Selected bond lengths (Å) and angles (°) of compound **3**.

Bond	Bond length
S1–S2	2.06(5)
S1–C15	1.80(1)
S2–C16	1.79(2)
O1–C13	1.35(2)
O4–C13	1.21(2)
O3–C18	1.20(2)
O2–C18	1.34(1)
C14–C15	1.41(2)
C15–C34	1.39(2)
C16–C17	1.41(2)
C17–C38	1.40(2)
Angle	**Bond angle**
S2–S1–C15	104.5(5)
S1–S2–C16	104.6(5)
O2–C18–O3	123.9(1)
O1–C13–O4	123.5(1)
C14–C15–C34	118.4(1)
C16–C17–C38	119.0(1)

Compound **3** is unusual in a few aspects. A search of the CSD shows that it is only the second example of a disulfide group appended by two phenyl rings [21] The first instance was reported by Kucsman and Kapovits and differs from the present compound in the length of the ester group [22]; being the methyl ester in comparison to the dodecyl equivalent presented herein. The dodecyl chain is also the longest benzoate ester reported in the CSD.

A feature of the structure reported by Kucsman and Kapovits is sulfur·oxygen non-bonded contacts, with interaction distances shorter than the sum of the van der Waals radii (3.25 Å). No such interactions are noted in the present structure. However, two other intermolecular interactions are observed. These are C–H···O interactions and S···π interactions. The C–H···O interactions link two adjacent molecules in the lattice into a dimeric supramolecular structure. The C–H, H···O and C···O distances measure 0.990, 2.592 and 3.324(2) Å, respectively. The C–H···O bond angle measures 130.77 °. The interaction distance is significantly shorter than the sum of the van der Waals radii of the interacting atoms, 0.128 Å shorter, suggesting that the interaction is genuine. In addition to the C–H···O interaction, the dimeric structure is further stabilised by S···π interactions. This class of interaction is well known and is significant in biomolecular systems, responsible for stabilising the folding of proteins and the associated quaternary structures. [23,24]. The distance between the centroid of the C36–C37–C17–C16–C35–C38 phenyl ring and S2 measures 3.765(1) Å [23]. This is comparable to the bond distances noted by ringer *et al.* in proteins which, although variable, typically measure 3.8 Å. The dimeric structure is shown in Figure 2.

3.3. Critical micelle concentration

The critical micelle concentrations of the dialkyl 2,2'-disulfanediyldibenzoateswere determined by fluorescence analysis using pyrene as the fluorescence probe. The onset of micellar formation when a surfactant is added to an aqueous solution of pyrene causes an abrupt decrease in the I_1/I_3 ratio, due to the solubilisation of pyrene molecule solubilized within the hydrophobic interior of micellar aggregates [25]. Figure 3 illustrates the fluorescence and CMC graph of compound **2**.

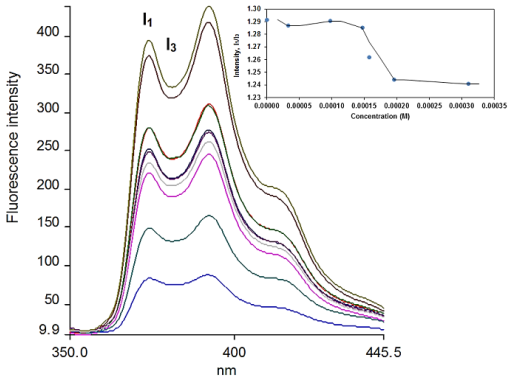


Figure 3. CMC determination of compound **2**.

Increase in lipophilicity of the dialkyl 2,2'-disulfanediyldi benzoates caused a decrease in the CMC (Table 3), which shows an enhancement in the ease of micelle formation. This is in line with previous studies which showed that CMC decreases according to Kleven's equation 3 [26]

$$\text{Log CMC} = A - Bn \quad (3)$$

where n is the number of carbon atom in the surfactant chain length; A and B are constants. For the dialkyl 2,2'-disulfanediyl dibenzoates series, the Kleven's equation was found to be

Table 3. Critical micelle concentration of compound **1-3**.

Compound	CMC (mM)
1	0.330
2	0.196
3	0.084

Table 4. Minimum inhibitory concentrations of compound **1-3**.

Compounds	Gram positive (mM)			Gram negative (mM)	
	S. aureus	*S. epidermidis*	*B. cereus*	*K. pneumoniae*	*E. coli*
1	4.72	2.36	4.72	2.36	9.43
2	1.95	1.07	2.13	1.07	3.89
3	0.04	0.06	1.95	0.02	0.12

Table 5. Binding of DPH to DPPC in the presence and absence of compounds

Compound	Chain length	K_1 (M^{-1})	n_1
3	12	4.9×10^5	0.012
CTAB	16	1.5×10^5	0.014
Streptomycin	16	3.0×10^6	0.015
Control *	-	3.0×10^6	0.032

* Binary system consisting of DPH and DPPC in the absence of the compound.

$$\text{Log CMC} = 0.7306 - 0.1486n \quad (4)$$

3.4. Antibacterial activity

The dialkyl 2,2'-disulfanediyldibenzoates displayed good antibacterial activity. Comparing the MIC of the three dialkyl-dithiodibenzoates (Table 4), it was found that their activity increases with an increase in chain length with the C_{12} derivative (**3**) displaying the best activity among the series. This is in line with previous studies where the increase in activity with chain length was associated with an increase in hydrophobic interaction with target cell membrane which causes disturbance in some membrane processes leading to cell death [27]. The compounds were also found to be more active towards Gram positive rather than Gram negative bacteria due to the presence of an additional lipopoly-saccharide membrane which lead to antibiotic resistance in gram negative strains [28].

Comparing the MIC values of compounds **1-3** with their CMC, it was found that compounds **1** and **2** act as antibacterial agent at a concentration much higher than their CMC. However, in the case of compound **3**, the MIC values were found to be mostly below the CMC which suggest that the antibacterial activity of compound **3** is mostly governed by their monomers rather than their micelles.

3.5. Phospholipid binding studies

In order to relate the antibacterial activity of the most active dialkyl 2,2'-disulfanediyldibenzoate, with its membrane affinity, the binding of compound **3** with DPPC was studied in the presence of DPH to measure its hydrophobic interactions with the phospholipid molecules (Figure 4).

Addition of compound **3** to the DPPC-DPH causes a decrease in the intensity, suggesting a competitive binding between the compounds **3** and DPH for DPPC.

The binding constants of DPH to DPPC in the presence of compound **3** as well as in the presence of the antibacterial agents CTAB and streptomycin obtained from previous report [29] is summarized in Table 5. The value of K_1 was found to be lower in the presence of compound **3** than that of the binary DPH-DPPC system, which suggest that compound **3** is able to displace the binding between DPH to DPPC since it binds in close proximity to the binding site of DPH. The binding constant observed in the presence of compound **3** was higher compared to that of CTAB, which might be due to a lower alkyl chain length, resulting in lower hydrophobic interaction with the phospholipid molecules. This might be the cause of the lower antibacterial activity of compound **3** compared to CTAB.

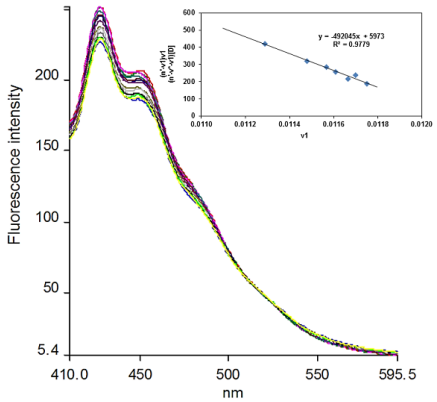


Figure 4. Binding of compound **3** with DPPC in the presence of DPH.

4. Conclusion

A series of dialkyl 2,2'-disulfanediyldibenzoates (compounds **1-3**) with chain length C_8 to C_{12} were successfully synthesized. We have reported the X-ray single crystal structure of compound **3**, which is the longest benzoate ester of diaryl benzoate ever reported till date. The compound **1-3** were found to possess good antibacterial activity, especially towards Gram positive bacteria. Their activity was found to be proportional to chain length, with the C_{12} derivative displaying the optimum activity. The antibacterial activity of the C_8-C_{10} derivative (compounds **1** and **2**) was governed by their micellar form, while the monomeric form of the C_{12} derivative (compound **3**) was found to be mostly responsible for its activity. Interaction of compound **3** with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) showed that the compound act as antibacterial agents by the involvement of hydrophobic interactions with phospholipid molecules of bacterial membrane.

Acknowledgements

The authors are highly thankful to the University of Mauritius for the necessary facilities and financial assistance.

Supplementary material

CCDC 1521391 contains the supplementary crystallographic data of compound **3**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre

via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found in the online version.

References

[1]. Ceylan, M.; Gürdere, M. B.; Karaman, I.; Gezegen, H. *Med. Chem. Res*. **2011**, *20*, 109-115.
[2]. Fletcher, J. M.; Huhues, R. A. *Tetrahedron Lett.* **2004**, *45*, 6999-7001.
[3]. Khan, K. M.; Taha, M.; Naz, F.; Khan, M.; Rahim, R.; Perveen, S. S.; Choudhary, I. M. *Med. Chem*. **2002**, *2*, 129-146.
[4]. Jhy-Jia, J.; Tsu-Chung, C.; Wen-Li, H.; Jinh-Min, H.; Ling-Yih, H. *Chem. Pharm. Bull.* **2003**, *511*, 1307-1310.
[5]. Vara Prasad, J. V. N.; Loo, J. A.; Boyer, F. E.; Stier, M. A.; Gogliotti, R. D.; Turner, W. J.; Harvey, P. J.; Kramer, M. R.; Mack, D. P.; Scholten, J. D.; Gracheck, S. J.; Domagala, J. M. *Bioorg. Med. Chem.* **1998**, *6*, 1707-1730.
[6]. Witrouw, M.; Balzarini, M. J.; Pannecouque, C.; Jhaumeer Laulloo, S.; ESTE, J. A.; Schols, D.; Cherepanov, P.; Scmit, J. C.; Debyser, Z.; Vandamme, A. M.; Desmyter, J.; Ramadas, S. R.; De Clercq, E. *Antimicrob. Agents. Chemother*. **1997**, *41*, 262-268.
[7]. Eshghi, H.; Rahimizadeh, M.; Zokaei, M.; Eshghi, S.; Faghihi, Z.; Tabasi, E.; Kihanyan, M. *Eur. J. Chem*. **2011**, *2*, 47-50.
[8]. Bonaccorsi, P.; Barattucci, A.; Papalia, T.; Criseo, G.; Faggio, C.; Romeo, O. *J. Sulfur Chem.* **2015**, *36*, 317-325.
[9]. Bhowon, M. G.; Jhaumeer laulloo, S.; Soukhee, N.; Allibacus, A.; Shiboo, V. *J. Coord. Chem*. **2007**, *60*(*12*), 1335–1343.
[10]. Jhaumeer Laulloo, S.; Bhowon, M. G.; Ravikumar, S.; Kalaiyarasi, A.; Raja, M.; Vijayakumar, V. *Int. J. Med. Med. Sci*. **2013**, *5*(*4*), 260-263.
[11]. Balgavy, P.; Devinsky, F. *Adv. Colloid. Interface. Sci*. **1996**, *66*, 23-63.
[12]. Joondan, N.; Jhaumeer Laulloo, S.; Caumul, P. *J. Surfactants. Deterg*. **2015**, *18*, 1095-1104.
[13]. Turos, E.; Revell, K. D.; Ramaraju, P.; Gergeres, D. A.; Greenhalgh, K.; Young, A.; Sathyanarayan, N.; Dickey, S.; Lim, D.; Alhamadsheh, M. M.; Reynolds, K. *Bioorg. Med. Chem.* **2008**, *16*, 6501-6508.
[14]. Nyamato, G. S.; Ojwach, S. O.; Akerman, M. P. *Dalton Trans*. **2016**, *45*, 3407-3416.
[15]. Bruker. APEX2, SAINT and SADABS. Bruker AXS Inc., Madison, Wisconsin, USA, 2012.
[16]. Sheldrick, G. M. *Acta Cryst.* **2015**, *C71*, 3–8.
[17]. Farrugia, L. J. *J. Appl. Cryst.* **2012**, *45*, 849-854.
[18]. Joondan, N.; Caumul, P.; Jhaumeer Laulloo, S. *J. Surfactants. Deterg*. **2016**. DOI: 10. 1007/s11743-016-1895-7.
[19]. Joondan, N.; Jhaumeer Laulloo, S.; Caumul, P. *Microbiol. Res*. **2014**, *169*, 675-685.
[20]. Bruno, I. J.; Cole, J. C.; Kessler, M.; Jie, L.; Motherwell, W. D. S.; Purkis, L. H.; Smith, B. R.; Taylor, R.; Cooper, R. I.; Harris, S. E.; Orpen, A. G. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 2133-2144.
[21]. Groom, C. R.; Bruno, I. J.; Lightfoot, M. P.; Ward, S. C. *Acta Cryst.* **2016**, *B72*, 171-179.
[22]. Kucsman, A.; Kapovits, L. *J. Mol. Struct*. **1984**, *125*, 331 – 347.
[23]. Ringer, A. L.; Seneko, A.; Sherrill, D. *Protein Science*, **2007**, *16*, 2216 – 2223.
[24]. Daeffler, K. N. M.; Lester, H. A.; Dougherty, D. A. *J. Am. Chem. Soc*. **2012**, *134*, 14890 – 14896.
[25]. Pineiro, C.; Novo, M.; Al-Soufi, W. *Adv. Colloid. Int. Sci*. **2015**, *215*, 1-12.
[26]. Klevens, H. B. *J. Am. Oil. Chem. Soc.* **1953**, *30*, 74-80.
[27]. Lindstedt, M.; Allenmark, S.; Thompson, R. A.; Edebo, L. *Antimicrob. Agents. Chemother*. **1990**, *34*, 1949-1954.
[28]. Campbell, N. 3rd ed, Benjamin Cummings Publishing, Redwood City, Calif, 1993.
[29]. Joondan, N.; Caumul, P.; Akerman, M.; Jhaumeer Laulloo, S. *Bioorg. Chem*. **2015**, *58*, 117-129.