

Synthesis and evaluation of inhibitory potentials of microbial biofilms and quorum-sensing by 3-(1,3-dithian-2-ylidene) pentane-2,4-dione and ethyl-2-cyano-2-(1,3-dithian-2-ylidene) acetate

Alfred Ngege Tamfu¹, Wafia Boukhedena², Sameh Boudiba², Samir Deghboudj², Ozgur Ceylan³

¹ University of Ngaoundere, Ngaoundere, Cameroon

² Larbi Tebessi University, Tebessa, Algeria

³ Mugla Sıtkı Kocman University, Mugla, Turkey

Corresponding author: Alfred Ngege Tamfu (macntamfu@yahoo.co.uk)

Received 15 June 2022 ♦ Accepted 19 August 2022 ♦ Published 7 November 2022

Citation: Ngege Tamfu A, Boukhedena W, Boudiba S, Deghboudj S, Ceylan O (2022) Synthesis and evaluation of inhibitory potentials of microbial biofilms and quorum-sensing by 3-(1,3-dithian-2-ylidene) pentane-2,4-dione and ethyl-2-cyano-2-(1,3-dithian-2-ylidene) acetate. Pharmacia 69(4): 973–980. <https://doi.org/10.3897/pharmacia.69.e87834>

Abstract

The virulence and resistance of pathogenic microorganisms are promoted by quorum-sensing (QS) mediated traits and biofilms. The development of antimicrobial agents which can reduce the incidence of microbial resistance by disrupting the establishment of biofilms and QS, constitute a suitable strategy to reduce the emergence of pathogenic strains that are resistant to antibiotics. In this study, 3-(1,3-dithian-2-ylidene) pentane-2,4-dione (1) and ethyl-2-cyano-2-(1,3-dithian-2-ylidene) acetate (2) were successfully synthesized and characterized using EIMS, ¹H NMR and ¹³C NMR techniques. On *S. aureus*, both compounds had MIC (minimal inhibitory concentrations) of 0.625 mg/mL while on *E. coli* and *C. albicans*, compound 2 showed higher activity than compound 1. All compounds inhibited formation of biofilms by *C. albicans* and *S. aureus* at sub-MIC with compound 1 being more active than compound 2. On *E. coli*, only compound 1 inhibited biofilm formation. Violacein production of violacein in *C. violaceum* CV12472 and quorum sensing in *C. violaceum* CV026 were inhibited indicating that the compounds could block signal production and reception. Anti-quorum sensing at sub-MIC concentrations revealed by inhibition zones were 13.0±0.5 mm and 8.0±0.5 mm at MIC and MIC/2 respectively for compound 1 and for compound 2, they were 11.5±0.4 mm and 7.5±0.0 mm at MIC and MIC/2 respectively. Concentration-dependent swarming motility was exhibited by both compounds with compound 1 slightly more active than compound 2. The results indicate that the organosulphur compounds could be suitable candidates for modern antibiotics.

Keywords

organosulphur compounds, 3-(1,3-dithian-2-ylidene) pentane-2,4-dione, ethyl-2-cyano-2-(1,3-dithian-2-ylidene) acetate, antimicrobial activity, antibiofilm, quorum-sensing inhibition

Introduction

Resistance of pathogenic bacteria to most antibiotics is a threat to both animal and human health throughout the world and globally accounting for a rising number of deaths every year (about 700,000 human lives annually worldwide) and it is estimated that about 10 million people could die every year from infectious diseases due to antimicrobial resistance by 2050, with accompanying financial costs of about hundred trillion U.S. dollars, indicating that it could have more magnitude than major diseases like malaria and HIV aids (Antimicrobial Resistance Collaborators 2022; Baekkeskov et al. 2022). The increasing and wide range of infections caused by parasites, bacteria, fungi and viruses becomes difficult to treat with common medicines as they quickly develop resistance to each new antibiotic that enters the market, thereby making search for new antibiotics and antimicrobial agents imperative (Prestinaci et al. 2015). Resistance to antibiotics of certain bacteria is primarily due to many factors including alteration of drug targets, impermeability of antibiotic into the microbial cells within colonies (formation of biofilms), genetic mutations, generation of resistance genes and their transfer through plasmids (Heidari et al. 2022). Under adverse and unfavorable conditions, microbes can mutate into forms that have higher resistance to drugs, making it difficult for the normal antimicrobial substances that may kill or inhibit bacteria and these class of antibiotics are progressively getting out of use since they cannot inhibit quorum-sensing and formation of biofilms (resistance factors) in bacteria (Tamfu et al. 2020b). Biofilms are bacterial clusters attached unto a surface and/or to each other and covered with a self-synthesized protective coating or matrix made up of substances like proteins and polysaccharides which shields the bacterial from antibiotics and host defense systems and also to survive harsh conditions and hunger (Vestby et al. 2020; Tamfu et al. 2020c). QS also considered as cell-to-cell networks of communication refers to the ability within bacterial communities to sense and respond rapidly to the changes in their environment such as cell density, facilitated by small secreted signaling molecules referred to as auto-inducers (AI) and many quorum sensing mediated factors, such as swarming and swimming motilities, violacein synthesis, production of exopolysaccharide and biosurfactants contributes to the ability to form biofilms and develop resistance in bacteria (Packiavathy et al. 2014; Tamfu et al. 2020a). Bacterial biofilms are responsible for almost 80% of severe and perpetual microbial infections on humans and bacterial cells living inside biofilms maybe 10 to about 1000 times more resistant to antimicrobial agents than their planktonic counterparts (Sharma et al. 2019). Recent developments of strategies such as disruption of biofilm and QS are gaining grounds and are used to combat the emergence of resistant microbial strains which are continuously constituting a major health issue across the globe (Ngenge et al. 2021). There is a need for the search of alternative antimicrobial drugs which are safer with greater efficacy by re-

searchers and this has motivated increasing investigations on herbal and synthetic products for the discovery of new anti-pathogenic therapeutics that might be less toxic and also capable of inhibiting QS, thereby reducing infections and at the same time controlling or avoiding development of resistance by bacteria (Tamfu et al. 2020a; Ceylan et al. 2020; Kocak et al. 2021). The situation has created an urgent and highly demanding necessity for new antibiotic therapies that can subdue the emergence of strains that are resistant to existing antibiotics and disrupt microbial cell-to-cell communication systems (QS) and elimination of biofilm formation which appears to be a suitable solution (Wang et al. 2019).

Some organosulfur compounds including thiosulfonates, trisulfides and benzylsulfonic acid derivatives have been described as antibacterial and antifungal agents, suggesting that sulfur containing compounds could have antimicrobial activity (Kim et al. 2006). This study targeted the syntheses of 3-(1,3-dithian-2-ylidene)pentane-2,4-dione and ethyl-2-cyano-2-(1,3-dithian-2-ylidene)acetate, and evaluation of their effects on microbial biofilms and quorum sensing.

Materials and methods

Synthesis and characterization of compounds 1 and 2

Ethyl-2-cyano-2-(1,3-dithian-2-ylidene)acetate (Compound 2) was prepared from potassium carbonate, K_2CO_3 , (42 g, 0.3 mol) and ethyl 2-cyanoacetate (0.1 mol), a suitable active methylene compound (AMC), were measured and mixed in 50 mL of DMF (dimethylformamide). A magnetic stirrer was used to homogenize reaction mixture followed by the addition of carbon disulfide (9 ml, 0.15 mol) at room temperature. 1,3-dibromopropane (0.12 mol) was added drop wise to the mixture and the stirring continued for about 10 min. The reaction mixture was allowed under the stirrer for further 7 h after which 500mL of ice-cold water were added. A yellow precipitate was formed and was filtered out and dried on a Whatman number 1 filter paper. The yield: 93%; The molar mass of the compound was determined to be $M = 229$ g/mol from which the molecular formula $C_9H_{11}NO_2S_2$ deduced. The melting point determined was $95^\circ C$. FT-IR spectra of compound 2 obtained recorded in solid state, ν (cm^{-1}): 1700 (C=O), 1246–1004 (C–O ester), 2206 (C≡N), 1437 (C=C). 1H NMR ($CDCl_3$, 400 MHz): δ_H ppm; 1.35 (t, 3H, CH_3-CH_2), 2.30 (m, 2H, CH_2), 3.00 (t, 2H, CH_2S), 3.10 (t, 2H, CH_2S), 4.30 (q, 2H, CH_2O). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 14.22 (s, $\underline{C}H_3-CH_2-O$), 23.36 (s, $S-\underline{C}H_2-CH_2-CH_2-S$), 28.99 (s, $S-\underline{C}H_2-CH_2-CH_2-S$), 61.26 (s, $CH_3-\underline{C}H_2$), 120.55 (s, $\underline{C}N$), 76.69 (s, $O-\underline{C}-C=C$), 165.56 (s, $O-\underline{C}=O$), 180.7 (s, $C=\underline{C}S_2$).

3-(1,3-dithian-2-ylidene)pentane-2,4-dione (Compound 1): pentane-2,4-dione, was used as the active methylene compound to which K_2CO_3 (21 g, 0.15 mol) and

5.2 mL (0.05 mol) of DMF were added and the mixture stirred using a magnetic stirrer. 4.5 mL of carbon disulfide (0.075 mol) were added and the stirring continued for 10 mins after which 1,3-dibromopropane (0.06 mol) was introduced in a drop wise manner for 20 min under continuous stirring. 250 mL of ice-cold water was introduced into the reaction mixture and continuously stirred for further 7 hours at room temperature. Orange crystals of compound **1** were obtained after the precipitate formed was filtered and purified through recrystallization in ethanol. The yield of the synthesis was 83%. The molecular formula of compound **1** was determined as $M = 216$ g/mol and the molecular formula derived from there was $C_9H_{12}O_2S_2$. The melting point of the compound **1** was 104 °C. The FT-IR spectra of compound **1** determined in the solid state, ν (cm^{-1}): 1630 cm^{-1} (C=O), 1725 cm^{-1} ($H_3C-C=O$), 1173–1234 cm^{-1} (C-S-C), 1415 cm^{-1} (C=C). 1H NMR ($CDCl_3$, 400 MHz): δ_H ppm; 2.32 (s, 6H, 2 CH_3), 2.25 (m, 2H, CH_2), 2.95 (t, 4H, 2 CH_2).

Antimicrobial assays

Microbial strains

The microorganisms used in this study are *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10239, *Candida tropicalis* ATCC 13803, *Pseudomonas aeruginosa* PA01 *Chromobacterium violaceum* CV12472 and *Chromobacterium violaceum* CV026.

Determination of Antimicrobial activity (MIC)

Minimal inhibitory concentrations (MICs) were evaluated using 96-well microplates by broth dilution method described by the Clinical and Laboratory Standards Institute (CLSI, 2006). MIC was the least concentration of the test compound in which there was no visible microbial growth. Mueller-Hinton broth was used as the medium and 5×10^5 colony-forming units (CFU)/mL of bacterial density was used. 100 μ L of microbial cell suspension were introduced into the wells of 96-well plates together with the test compounds at final concentrations of 5.0, 2.5, 1.25, 0.625, 0.312, 0.156 mg/mL. The microplates were then incubated at 37 °C overnight and MIC values deduced based on optical densities.

Assay of antibiofilm activity of test compounds

The antibiofilm effect of the compounds at MIC and sub-MIC concentrations (1, 1/2, 1/4 and 1/8 MIC) on test pathogens were evaluated using a microplate biofilm method (Merritt et al. 2005). 1% of overnight bacterial cultures were introduced into 200 μ L of Tryptose-Soy Broth (TSB) containing 0.25% of glucose and with or without the test compounds and incubated at 37 °C for 48 hours and then wells of the plates were carefully emptied and rinsed with

distill water so as to remove the planktonic microbial cells. The control wells contained only broth and bacterial cells. The bacterial cells within the established biofilms were subsequently stained at room temperature by introducing 0.1% solution of crystal violet for about 10 minutes after which the crystal violet solution was pipetted out. The wells of the plates were then filled with 200 μ L of either ethanol (70%) or glacial acetic acid (33%) and then shaken gently. The optical densities (OD) were then recorded at 550 nm using a Thermo Scientific Multiskan FC, Vantaa, Finland spectrophotometer. The antibiofilm activity expressed as percentage inhibition of the test compounds were deduced using the formula:

$$\text{Biofilm inhibition(\%)} = \frac{OD_{550\text{Control}} - OD_{550\text{Sample}}}{OD_{550\text{Control}}} \times 100$$

Bioassay for quorum-sensing inhibition (QSI) on *C. violaceum* CV026

The quorum-sensing inhibition of the compounds were assayed as described elsewhere (Koh and Tham 2011) with some very little modifications. 100 μ L of overnight fresh cultures of CV026 were mixed with 5 mL of milliliters of warmly prepared molten soft agar prepared by mixing in 200 mL deionized H_2O , 1.0 g NaCl, 1.3 g agar and 2.0 g tryptone, followed by addition 20 μ L of exogenous acyl-homoserine lactone (C_6 HSL at 100 μ g/mL concentration). The molten agar mixture containing the CV026 bacterial cells were poured gently as overlay onto the surface of solidified Luria-Bertani Agar (LBA) plates. 5 mm diameter wells were made on the plates after solidification and subsequently filled with 50 μ L of sterilized MIC and sub-MIC concentrations of test compounds. The plates were incubated at 35 °C for 3 days after which they were observed for anti-QS activity in which a cream-colored circle formed around the well on the purple surface of actively growing CV026 bacteria indicated QSI and the diameters were recorded and given in mm. For each sample, three parallel assays were conducted and QSI was the average of the inhibition zones.

Evaluation of violacein inhibition in *C. violaceum* CV12472

Each of the test compounds was evaluated for its ability to inhibit the synthesis of violacein by *C. violaceum* ATCC 12472 in a qualitative assay as described previously (Tamfu et al. 2020a). 10 μ L of overnight fresh cultures of CV12472 (0.4 OD at 600 nm) were mixed with 170 μ L of LB broth in sterilized microplates and 20 μ L of test compounds MIC and sub-MIC concentrations. Assay in which the compound was not added (LB broth and CV12472) served as positive control. Test plates were incubated for 24 hrs at 35 °C after which the absorbances were read at 585 nm to determine any reduction of violacein pigment with respect to the control. Violacein inhibition expressed as percentage inhibition was deduced from the formula:

$$\text{Violacein inhibition(\%)} = \frac{OD_{585\text{control}} - OD_{585\text{sample}}}{OD_{585\text{control}}} \times 100$$

Inhibition of swarming motility on *P. aeruginosa* PA01

The determination of swarming movement in *P. aeruginosa* PA01 was done according to the method described elsewhere (Packiavathy et al. 2012). Summarily, plates consisting of swarming agar were prepared by mixing 0.5% agar, 0.5% NaCl, 0.5% D-glucose and 1% peptone and MIC, MIC/2 and MIC/4 concentrations of test compounds which were added before solidification. 5 μ L of *P. aeruginosa* PA01 overnight fresh cultures were point-inoculated on the center of each plate and plates without compounds were used as controls. Each plate was incubated at 37 °C and after 24 hrs, swarming migration was determined by measuring the swarming front diameters and percentage reduction in swarming calculated with respect to the control plates.

Results

Structures of compounds 1 and 2

The structures of compounds 1 and 2 are given in Fig. 1. The compounds were prepared from their respective active methylene compounds by first reacting with a base which deprotonates the methylene proton. The carbanion formed is stabilized through resonance by the carbonyl groups as in compound 1 or the carbonyl of ester group and the cyanide functional group as in compound 2. The carbanion then attacks the carbondisulphide, followed by the formation of the dithiol intermediate which then reacts with the 1,3-dibromide to give the final product as shown on Figs 2, 3.

Antimicrobial activity

Some sulfur-containing compounds have been used as antibiotics to treat infectious diseases especially for treating various skin diseases. The synthesized dithiane derivatives showed interesting antimicrobial property against *C. albicans*, *E. coli* and *S. aureus*. The antimicrobial activity of the two compounds, 3-(1,3-dithian-2-ylidene) pentane-2,4-dione and ethyl-2-cyano-2-(1,3-dithian-2-ylidene) acetate, are reported as MIC values, which is defined as the lowest concentration of compound for which no growth

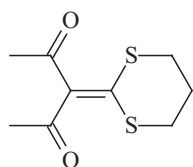
of bacterial cells was visible and the results are reported on Table 1. On the Gram positive bacterium *S. aureus*, both compounds had the same antimicrobial activity with MIC values of 0.625 mg/mL. On the Gram negative bacterium *E. coli*, compound 1 was less active (MIC = 1.25 mg/mL) than compound 2 (MIC = 0.625 mg/mL) and equally on the yeast *C. albicans*, where compound 1 exhibited a MIC of 0.625 mg/mL compared to compound 2 with MIC of 0.312 mg/mL. It could be seen that the tested microorganisms exhibited more susceptibility to compound 2 than to compound 1.

Antibiofilm activity

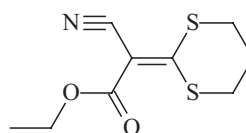
Biofilm is composed of sessile microbial cells living within a self-produced polymeric matrix that offers a protective shield to them and reduces the penetration of antibiotics. The effectiveness of each of the compounds to inhibit formation of bacterial biofilms was evaluated through the crystal violet staining method at MIC and sub-MIC concentrations of the compounds and the obtained results presented on Table 1 as percentage inhibitions. On *S. aureus*, a Gram-positive bacteria, the inhibition of biofilm for compound 1 varied from 60.12 \pm 1.78% at MIC to 11.70 \pm 0.28% at MIC/4 and for compound 2, it varied from 43.12 \pm 0.86% at MIC to 4.21 \pm 0.15% at MIC/4. On *E. coli*, a Gram-negative bacteria, only compound 1 inhibited biofilm formation with percentage inhibition of 15.45 \pm 0.62% at MIC to 5.4 \pm 0.12% at MIC/2. Both compounds inhibited biofilm formation by the yeast *C. albicans* with percentage inhibitions of 22.23 \pm 0.52% and 18.7 \pm 0.25% for compound 1 and compound 2 respectively at MIC and 6.5 \pm 0.15% and 3.6 \pm 0.11% for compounds 1 and 2 respectively at MIC/2.

Violacein synthesis inhibition in *C. violaceum* CV12472 and inhibition of quorum sensing in *C. violaceum* CV026

Violacein is a bisindole purple pigment synthesized by *Chromobacterium violaceum* which is a Gram-negative bacteria. The two strains used for the study reported here are *C. violaceum* CV12472 which is capable of producing violacein and *C. violaceum* CV026 (mutant strain) which produces violacein only when an acylhomoserine lactone is supplied to it externally. Despite the condition under



Compound 1
3-(1,3-dithian-2-ylidene) pentane-2,4-dione



Compound 2
Ethyl cyano (1,3-dithian-2-ylidene) acetate

Figure 1. Structures of compounds 1 and 2.

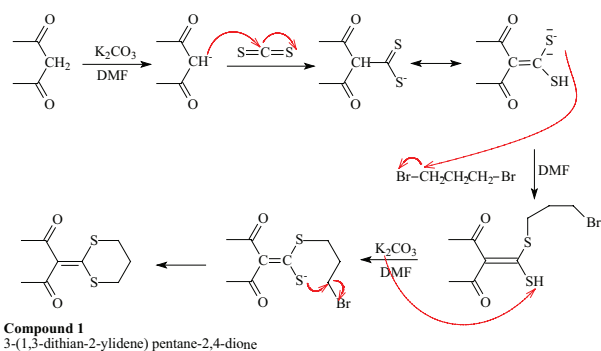


Figure 2. Schematic representation of the synthesis of 3-(1,3-dithian-2-ylidene) pentane-2,4-dione (Compound 1).

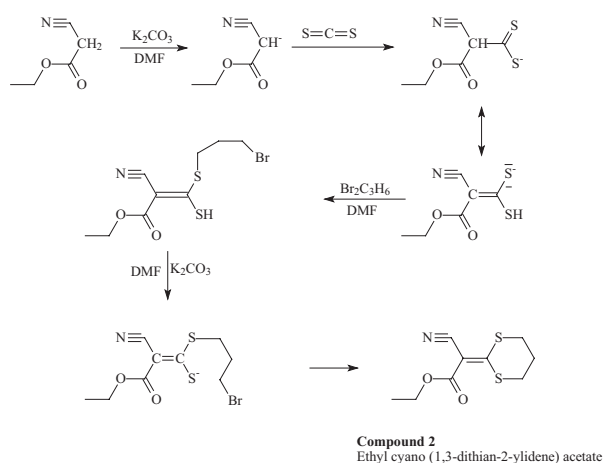


Figure 3. Schematic representation of the synthesis of ethyl cyano (1,3-dithian-2-ylidene) acetate (Compound 2).

which the *C. violaceum* produces violacein, the process is controlled by quorum sensing, and since it is easy to measure the absorbance of the violacein pigment, it has become an important indicator used in evaluation of quorum sensing in bacteria. The violacein inhibitory potential of the compounds were evaluated at sub-MIC and MIC concentrations on *C. violaceum* CV12472 and the percentage inhibitions are recorded on Table 2. Both compounds inhibited violacein production at 100% at MIC. However, the activity reduces in a concentration-dependent manner to 15.1±1.1% at MIC/8 for compound 1 and 16.8±0.5% at MIC/16 for compound 2. It was observed that compound 2 had a greater violacein inhibition potential than compound 1.

Prior to this, the MIC of the honey samples were determined so that the MIC values of compound 1 and compound 2 were 0.312 mg/mL and 0.625 mg/mL respectively on *C. violaceum* CV026. The anti-quorum sensing activity is determined at sub-MIC and MIC concentrations. On the test plates, a cream-colored circular halo around a well against a purple coloured surface of activate CV026 bacteria on the plate indicated that QS was inhibited (Alfred et al. 2020) and the diameters of each zone of QS inhibition were measured and presented in Table 3. The QSI zones were 13.0±0.5 mm and 8.0±0.5 mm at MIC and MIC/2

Table 1. MIC and Anti-biofilm activity (percentage inhibition) results of test samples.

Microorganisms	Sample codes		
	Compound 1	Compound 2	
	MIC (mg/mL)		
<i>S. aureus</i>	0.625	0.625	
<i>E. coli</i>	1.25	0.625	
<i>C. albicans</i>	0.625	0.312	
Biofilm inhibition (%)			
<i>S. aureus</i>	MIC	60.12±1.78	43.12±0.86
	MIC/2	25.38±0.68	15.69±0.38
	MIC/4	11.70±0.28	4.21±0.15
	MIC/8	-	-
<i>E. coli</i>	MIC	15.45±0.62	-
	MIC/2	5.4±0.12	-
	MIC/4	-	-
	MIC/8	-	-
<i>C. albicans</i>	MIC	22.23±0.52	18.7±0.25
	MIC/2	6.5±0.15	3.6±0.11
	MIC/4	-	-
	MIC/8	-	-

-: no inhibition

Table 2. Inhibition of violacein production in *C. violaceum* CV12472 by test samples.

Sample code	MIC (mg mL)	Violacein inhibition (%)				
		MIC	MIC/2	MIC/4	MIC/8	MIC/16
Compound 1	0.312	100±0.00	81.3±1.0	38.1±0.10	15.1±1.1	-
Compound 2	0.312	100±0.00	100±0.00	67.1±1.50	35.4±0.9	16.8±0.5

-: no inhibition

Table 3. Quorum sensing inhibition zones in *C. violaceum* CV026 by test samples.

Sample code	MIC (mg mL)	Anti-quorum sensing inhibition zones (mm)			
		MIC	MIC/2	MIC/4	MIC/8
Compound 1	0.312	13.0±0.5	8.0±0.5	-	-
Compound 2	0.625	11.5±0.4	7.5±0.0	-	-

-: no inhibition

respectively for compound 1 and for compound 2, anti-QS zones were 11.5±0.4 mm and 7.5±0.0 mm at MIC and MIC/2 respectively. No QSI was visible below MIC/2.

Inhibition of swarming motility in *P. aeruginosa* PA01

Motility of microorganisms is one of the QS-mediated processes and it contributes to biofilm formation in bacteria. The test compounds reduced swarming movements in *P. aeruginosa* PA01 evaluated at three concentrations MIC, ½ MIC and ¼ MIC in a concentration-dependent manner and results were expressed as percentage inhibition as shown in Table 4. The swarming inhibition percentages varied from 53.1±1.6% at MIC to 17.1±0.4% at MIC/4 for compound 1 and varied from 51.0±0.2% at MIC to 15.5±0.1% at MIC/4 for compound 2. Compound 1 showed a slightly higher swarming motility inhibition than compound 2.

Table 4. Swarming motility inhibition on *P. aeruginosa* PA01 by test samples.

Sample code	MIC (mg/ mL)	Swarming inhibition (%)		
		MIC	MIC/2	MIC/4
Compound 1	2.5	53.1±1.6	32.7±0.5	17.1±0.4
Compound 2	2.5	51.0±0.2	30.5±0.8	15.5±0.1

-.: no inhibition

Discussion

The compounds exhibited antimicrobial activity and showed considerable MIC values against pathogenic microbes. Compound 2 had better antimicrobial activity than compound 1 and this could be attributed to the presence of cyanide function in compound 2 which is a nitrile. The toxic nature of most of the nitriles towards aerobic organisms and microorganisms results from the cyanide they release during their degradation process and this cyanide can act as an inhibitor of cytochrome C oxidase and some metalloenzymes (Egelkamp et al. 2019; Anand et al. 2020). However, the presence of sulfur in these compounds could also contribute to improving antimicrobial activity because the activity of *Allium* and *Brassica* vegetables against fungi, Gram-negative bacteria and Gram-positive bacteria was attributed to sulfur-containing compounds (Kyung and Lee 2007). Several organosulfur compounds isolated from roots of *Petiveria alliacea* L. showed interesting antibacterial and antifungal activities (Kim et al. 2006). Organosulfur compounds therefore have good antibacterial, antifungal and antiviral activities (Sagdic and Tornuk 2012). Several isothiocyanate compounds from *Raphanus sativus* L. Brassicaceae which is used in treating microbial infections, showed antibacterial activity (Jadoun et al. 2016). Integrating sulphur within the structures of synthesized compounds for example synthetic polymers could contribute in ameliorating the antibacterial activity of such compounds (Smith et al. 2020). Antimicrobial agents which only inhibit growth or try to kill bacteria are getting out of use as they are faced with resistance after poor usage. Novel antimicrobial agents with new modes of action capable of inhibiting biofilm formation and quorum sensing networks in bacteria are of great interest. Microbial biofilms are well-structured and sessile communities growing within an extracellular polymeric matrix and can develop on various living and nonliving surfaces including medical devices such as prosthesis and (Jamal et al. 2018). The polymeric matrix protects the biofilm communities from antimicrobial host defenses and antibiotic drugs (Karygianni et al. 2020). The fact that compound 1 and 2 were able to inhibit biofilm formation in some bacteria at low concentrations (sub-MIC concentrations) indicates a great potential of these types of compounds in eliminating resistance and reducing the virulence factors of pathogenic bacteria. Organosulfur compounds exhibit several properties against a variety of microorganisms including antitoxin, bactericidal, antibiofilm and anti-quorum sensing activities and the reactive organosulfur compounds are able to form disulfide linkages with the free

sulfhydryl groups of some enzymes causing a disintegration of the bacterial membrane (Bhatwalkar et al. 2021). In one study, all synthesized organosulfur compounds showed good antibacterial and antibiofilm activities and it was proposed that the sulfur-containing thiazolidione scaffold was evidently a good chemotype that could be explored for the development of antibiofilm therapeutics (Pan et al. 2010). During unfavorable conditions such as presence of antibiotics and scarcity of nutritive substances, most pathogenic microbes will organize themselves into self-organized colonies and build a protective and highly resistant and impermeable shield called biofilm that will help to keep their communities protected and enable them to stay viable and this will allow them to be able to regrow when conditions become favorable again and continue to cause harm their host (Tamfu et al. 2022a, b).

The matrix that envelopes the biofilm cells contributes in cell-to-cell communicative and signaling networks between the cells with the microbial colonies and this helps in decision-making and coordinative actions and this process is mediated through quorum sensing (Khatoun et al. 2018). The disruption of QS networks can cause the subsequent growth inhibition of the bacterial cells inside the polymeric biofilm matrix and can equally trigger the disintegration of biofilms that were already established through a process called quorum quenching, and thereby providing a good strategy for developing potential microbial treatments (Oppenheimer-Shaanan et al. 2013; Puiu et al. 2017). Both compounds were able to inhibit violacein synthesis in *C. violaceum* CV12472 and also block quorum-sensing in *C. violaceum* CV026 despite the external supply of an AHL to the latter. The *C. violaceum* CV12472 strain is able to produce its violacein and since the production of violacein was inhibited, this suggests that the compounds could block signal emission since violacein is a signal molecule (Tamfu et al. 2021). Also, since *C. violaceum* CV026 cannot produce AHL on its own, an external AHL was supply to it but zones of non-production of violacein were observed indicating that the compounds equally disrupted signal reception within the anti-QS zones (Tamfu et al. 2020b). The inhibition of violacein production, quorum sensing, biofilm formation and swarming motility which are controlled by QS are suitable strategies of overcoming microbial resistance and a biocontrol method to reduce the spread of notorious infections and this can be done by using and some natural compounds, synthesized compounds, polymers, nanomaterials and natural products which have shown some promising effects (Ceylan et al. 2020; Boudiba et al. 2021; Kocak et al. 2021; Popova et al. 2021; Tamfu et al. 2022c). Flagellum-mediated motility such as swarming movement serves the bacteria *Pseudomonas aeruginosa* PA01 to move to surface and attach themselves before further spreading and colonization of the surface (Ngeenge Tamfu et al. 2021). The swarming movements enable bacteria to colonize surfaces and this is a quorum-sensing mediated step necessary for the establishment of biofilms on surfaces, leading to the spread of infections and microbial resistance (Beddiar et al. 2021; Ngeenge et al. 2021). *Pseudomonas aeruginosa* is one of the

most pathogenic bacteria causing infections with high severity and virulence and the ability of compounds 1 and 2 to inhibit the swarming motility of this bacteria and its subsequent biofilm establishment indicates that these compounds could be used to protect equipment and surfaces from being colonized by pathogenic microbes and subsequently establishing sessile biofilms.

Conclusion

Antimicrobial resistance resulting mainly from genetic mutations, inappropriate or poor usage of antibiotics constitutes a major global health burden since various pathogenic bacteria, viruses and fungi are no longer susceptible to antibiotics designed to kill them. Various pathogens are now able to protect themselves from the substances of the host defense system, antibiotics and disinfectants. Microbial resistance is regulated by quorum sensing system (cell-to-cell communication network) which accounts for the regulation of biofilm formation, virulence factors, sporulation, toxin production, motility and drug resistance. Conventional antibiotics, designed to inhibit or kill bacteria are faced with resistance over time and are falling out of use, and therefore there is need to find new antimicrobial agents that can target quorum-sensing systems responsible for regulating the expression of virulence

factors as a strategy to reduce the emergence of bacterial resistance. In this study, two organosulfur compounds, 3-(1,3-dithian-2-ylidene) pentane-2,4-dione (compound 1) and ethyl-2-cyano-2-(1,3-dithian-2-ylidene) (compound 2) were synthesized and their antibiofilm and anti-quorum sensing activities evaluated. The results showed antimicrobial activity of both compounds supporting the antimicrobial nature of organosulfur compounds. Antibiofilm and anti-quorum sensing potentials were exhibited by both compounds, indicating that these compounds could find application as new generation antibiotics capable of circumventing microbial resistance.

Authors' contributions

ANT, WB, SB and SD: Methodology, Formal analysis, Investigation, Writing original draft. ANT, WB, SB, OC and SD: Materials, Resources, Supervision and editing. ANT, WB, SB, SD and OC: Conceptualization, Correction and Editing. All authors read and approved the final manuscript.

Acknowledgements

The authors are grateful to Larbi Tebessi University and Mugla Sitki Kocman University for material support.

References

- Alfred TN, Ceylan O, Kucukaydin S, Olmez OT, Godloves CF, Sylvain SK, Yeskaliyeva B, Duru ME, Ozturk M (2020) HPLC-DAD and GC-MS characterization of Cameroonian honey samples and evaluation of their antibiofilm, anti-quorum sensing and antioxidant activities. *Bulletin of Environment, Pharmacology and Life Sciences* 9(10): 132–142.
- Anand A, Chinchilla D, Tan C, Mène-Saffrané L, L'Haridon F, Weisskopf L (2020) Contribution of hydrogen cyanide to the antagonistic activity of *Pseudomonas* strains against *Phytophthora infestans*. *Microorganisms* 8(8): 1144. <https://doi.org/10.3390/microorganisms8081144>
- Antimicrobial Resistance Collaborators (2022) Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 399(10325): 629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)
- Baekkeskov E, Rubin O, Munkholm L, Zaman W (2022) Antimicrobial resistance as a global health crisis. *Oxford research encyclopedia of politics*. Retrieved 16 Feb., 1–23. <https://oxfordre.com/politics/view/10.1093/acrefore/9780190228637.001.0001/acrefore-9780190228637-e-1626>
- Beddiar H, Boudiba S, Benahmed M, Tamfu AN, Ceylan Ö, Hanini K, Kucukaydin S, Elomri A, Bensouici C, Laouer H, Akkal S, Boudiba L, Dinica PM (2021) Chemical composition, anti-quorum sensing, enzyme inhibitory, and antioxidant properties of phenolic extracts of *Clinopodium nepeta* L. Kuntze. *Plants* 10: 1955. <https://doi.org/10.3390/plants10091955>
- Bhatwalkar SB, Mondal R, Krishna SBN, Adam JK, Govender P, Anupam R (2021) Antibacterial properties of organosulfur compounds of garlic (*Allium sativum*). *Frontiers in Microbiology* 12: 613077. <https://doi.org/10.3389/fmicb.2021.613077>
- Boudiba S, Tamfu AN, Berka B, Hanini K, Hioun S, Allaf K, Boudiba L, Ceylan O (2021) Anti-quorum sensing and antioxidant activity of essential oils extracted from *Juniperus* species, growing spontaneously in Tebessa Region (East of Algeria). *Natural Product Communications* 16(6): 1–11. <https://doi.org/10.1177/1934578X211024039>
- Ceylan O, Tamfu AN, Doğaç Yİ, Teke M (2020) Antibiofilm and anti-quorum sensing activities of polyethylene imine coated magnetite and nickel ferrite nanoparticles. *3 Biotech* 10(12): 513. <https://doi.org/10.1007/s13205-020-02509-6>
- CLSI (Clinical Laboratory Standards Institute) (2006) *Quality Control Minimal Inhibitory Concentration (MIC) Limits for Broth Dilution and MIC Interpretative Breakpoints (M27-s2)*. Wayne, Pennsylvania, USA.
- Egelkamp R, Zimmermann T, Schneider D, Hertel R, Daniel R (2019) Impact of nitriles on bacterial communities. *Frontiers in Environmental Science* 7: 103. <https://doi.org/10.3389/fenvs.2019.00103>
- Heidari R, Sheikh AF, Hashemzadeh M, Farshadzadeh Z, Salmanzadeh S, Saki M (2022) Antibiotic resistance, biofilm production ability and genetic diversity of carbapenem-resistant *Pseudomonas aeruginosa* strains isolated from nosocomial infections in southwestern Iran. *Molecular Biology Reports* 49: 3811–3822. <https://doi.org/10.1007/s11033-022-07225-3>
- Jadoun J, Yazbak A, Rushrush S, Rudy A, Azaizeh H (2016) Identification of a new antibacterial sulfur compound from raphanus sativus seeds. *Evidence-Based Complementary and Alternative Medicine* Vol. 2016: 9271285. <https://doi.org/10.1155/2016/9271285>
- Jamal M, Ahmad W, Andleeb S, Jalil F, Imran M, Nawaz MA, Hussain T, Ali M, Rafiq M, Kamil MA (2018) Bacterial biofilm and associated

- infections. *Journal of the Chinese Medical Association* 81(1): 7–11. <https://doi.org/10.1016/j.jcma.2017.07.012>
- Karygianni L, Attin T, Thurnheer T (2020) Combined DNase and proteinase treatment interferes with composition and structural integrity of multispecies oral biofilms. *Journal of Clinical Medicine* 9: 983. <https://doi.org/10.3390/jcm9040983>
- Khatoun Z, McTiernan CD, Suuronen EJ, Mah TE, Alarcon EI (2018) Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon* 4(12): e01067. <https://doi.org/10.1016/j.heliyon.2018.e01067>
- Kim S, Kubec R, Musah RA (2006) Antibacterial and antifungal activity of sulfur-containing compounds from *Petiveria alliacea* L. *Journal of Ethnopharmacology* 104(1–2): 188–192. <https://doi.org/10.1016/j.jep.2005.08.072>
- Kocak G, Tamfu AN, Bütün V, Ceylan O (2021) Synthesis of quaternary piperazine methacrylate homopolymers and their antibiofilm and anti-quorum sensing effects on pathogenic bacteria. *Journal of Applied Polymer Science* 138: e50466. <https://doi.org/10.1002/app.50466>
- Koh KM, Tham FY (2011) Screening of traditional Chinese medicinal plants for quorum-sensing inhibitors activity. *Journal of Microbiology, Immunology and Infection* 44: 144–148. <https://doi.org/10.1016/j.jmii.2009.10.001>
- Kyung KH, Lee YC (2007) Antimicrobial activities of sulfur compounds derived from s-alk(en)yl-l-cysteine sulfoxides in allium and brassica. *Food Reviews International* 17(2): 183–198. <https://doi.org/10.1081/FRI-100000268>
- Merritt JH, Kadouri DE, O'Toole GA (2005) Growing and analyzing static biofilms. *Current Protocols in Microbiol.* John Wiley & Sons, Inc. <https://doi.org/10.1002/9780471729259.mc01b01s00>
- Ngege AT, Ceylan O, Fru GC, Arab Y, Emin DM, Ozturk M (2021) Antimicrobial, antibiofilm, anti-quorum sensing and motility inhibition activities of essential oil from seeds of food spice *Xylopiya aethiopia* (Dunal) A. Rich. on some pathogenic bacteria. *Research Journal of Biotechnology* 16(6): 68–76.
- Ngege Tamfu A, Kucukaydin S, Ceylan O, Emin DM (2021) Evaluation of enzyme inhibition and anti-quorum sensing potentials of *Melaleuca alternifolia* and *Citrus sinensis* essential oils. *Natural Product Communications* 16(9): 1–8. <https://doi.org/10.1177/1934578X211044565>
- Oppenheimer-Shaanan Y, Steinberg N, Kolodkin-Gal I (2013) Small molecules are natural triggers for the disassembly of biofilms. *Trends in Microbiology* 21(11): 594–601. <https://doi.org/10.1016/j.tim.2013.08.005>
- Packiavathy IA, Priya S, Pandian SK, Ravi AV (2014) Inhibition of biofilm development of uropathogens by curcumin - an anti-quorum sensing agent from *Curcuma longa*. *Food Chemistry* 148: 453–460. <https://doi.org/10.1016/j.foodchem.2012.08.002>
- Packiavathy IASV, Agilandeswari P, Musthafa KS, Pandian SK, Ravi AV (2012) Antibiofilm and quorum sensing inhibitory potential of *Cuminum cyminum* and its secondary metabolite methyl eugenol against Gram negative bacterial pathogens. *Food Research International* 45: 85–92. <https://doi.org/10.1016/j.foodres.2011.10.022>
- Pan B, Huang RZ, Han SQ, Qu D, Zhu ML, Wei P, Ying HJ (2010) Design, synthesis, and antibiofilm activity of 2-arylimino-3-aryl-thiazolidine-4-ones. *Bioorganic & Medicinal Chemistry Letters* 20(8): 2461–2464. <https://doi.org/10.1016/j.bmcl.2010.03.013>
- Popova M, Gerginova D, Trusheva B, Simova S, Tamfu AN, Ceylan O, Clark K, Bankova V (2021) A preliminary study of chemical profiles of honey, cerumen, and propolis of the African stingless bee *Meliponula ferruginea*. *Foods* 10: 997. <https://doi.org/10.3390/foods10050997>
- Prestinaci F, Pezzotti P, Pantosti A (2015) Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health* 109(7): 309–318. <https://doi.org/10.1179/2047773215Y.0000000030>
- Puiu RA, Dolete G, Ene AM, Nicoară B, Vlăsceanu GM, Holban AM, Grumezescu AM, Bolocan A (2017) Properties of biofilms developed on medical devices, biofilms and implantable medical devices. *Infection and Control*, 25–46. <https://doi.org/10.1016/B978-0-08-100382-4.00002-2>
- Sagdic O, Tornuk F (2012) Antimicrobial properties of organosulfur compounds. In: Patra A (Eds) *Dietary Phytochemicals and Microbes*. Springer, Dordrecht, 127–156. https://doi.org/10.1007/978-94-007-3926-0_4
- Sharma D, Misba L, Khan AU (2019) Antibiotics versus biofilm: an emerging battleground in microbial communities. *Antimicrobial Resistance & Infection Control* 8: 76. <https://doi.org/10.1186/s13756-019-0533-3>
- Smith JA, Mulhall R, Goodman S, Fleming G, Allison H, Raval R, Hasell T (2020) Investigating the Antibacterial Properties of Inverse Vulcanized Sulfur Polymers. *ACS Omega* 5(10): 5229–5234. <https://doi.org/10.1021/acsomega.9b04267>
- Tamfu AN, Ceylan O, Fru GC, Ozturk M, Duru ME, Shaheen F (2020a) Antibiofilm, anti-quorum sensing and antioxidant activity of secondary metabolites from seeds of *Annona senegalensis*, Persoon. *Microbial Pathogenesis* 144: 104191. <https://doi.org/10.1016/j.micpath.2020.104191>
- Tamfu AN, Ceylan O, Kucukaydin S, Duru ME (2020b) HPLC-DAD phenolic profiles, antibiofilm, anti-quorum sensing and enzyme inhibitory potentials of *Camellia sinensis* (L.) O. Kuntze and *Curcuma longa* L. *LWT - Food Science and Technology* 133: 110150. <https://doi.org/10.1016/j.lwt.2020.110150>
- Tamfu AN, Ceylan O, Kucukaydin S, Ozturk M, Duru ME, Dinica RM (2020c) Antibiofilm and enzyme inhibitory potentials of two Annonaceous food spices, African pepper (*Xylopiya aethiopia*) and African nutmeg (*Monodora myristica*). *Foods* 9(12): 1768. <https://doi.org/10.3390/foods9121768>
- Tamfu AN, Kucukaydin S, Ceylan O, Sarac N, Duru EM (2021) Phenolic composition, enzyme inhibitory and anti-quorum sensing activities of cinnamon (*Cinnamomum zeylanicum* Blume) and Basil (*Ocimum basilicum* Linn). *Chemistry Africa* 4: 759–767. <https://doi.org/10.1007/s42250-021-00265-5>
- Tamfu AN, Kucukaydin S, Quradha MM, Ceylan O, Ugur A, Emin Duru M (2022a) Ultrasound-Assisted extraction of *Syringa vulgaris* Mill., *Citrus sinensis* L. and *Hypericum perforatum* L.: phenolic composition, enzyme inhibition and anti-quorum sensing activities. *Chemistry Africa* 5: 237–249. <https://doi.org/10.1007/s42250-022-00315-6>
- Tamfu AN, Munvera AM, Botezatu AVD, Talla E, Ceylan O, Fotsing MT, Mbafor JT, Shaheen F, Dinica RM (2022b) Synthesis of benzoyl esters of β -amyryn and lupeol and evaluation of their antibiofilm and antidiabetic activities. *Results in Chemistry* 4: 100322. <https://doi.org/10.1016/j.rechem.2022.100322>
- Tamfu AN, Ceylan O, Cârâc G, Talla E, Dinica RM (2022c) Antibiofilm and anti-quorum sensing potential of cycloartane-type triterpene acids from cameroonian grassland propolis: phenolic profile and antioxidant activity of crude extract. *Molecules* 27(15): 4872. <https://doi.org/10.3390/molecules27154872>
- Vestby LK, Grønseth T, Simm R, Nesse LL (2020) Bacterial biofilm and its role in the pathogenesis of disease. *Antibiotics (Basel)* 9(2): 59. <https://doi.org/10.3390/antibiotics9020059>
- Wang W, Dongxiang L, Xiaoqin H, Huixiang Y, Ziwen Q, Liting Z, Qin L, Yu S, Yingxiang W, Shaohua W, Chao Y, Yongyu L (2019) Study on antibacterial and quorum-sensing inhibition activities of *cinnamomum camphora* leaf essential oil. *Molecules* 24: 3792. <https://doi.org/10.3390/molecules24203792>