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RESEARCH ARTICLE

Synthesis of Some Benzothiazolone Derivatives and Their Effects on Quorum Sensing

Yasemin DÜNDAR[*°], Meryem Burcu KÜLAHCI[**], Özge KUYRUKÇU ÖZTÜRK[***], Ebru BEYZİ[****]

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SUMMARY

Quorum sensing (QS), which enables bacteria to communicate by sensing their population density and, in particular, to regulate biofilm formation and virulence factor production in pathogenic bacteria, has emerged as a novel target for the control of bacterial infections. In this study, the QS inhibitory effects of benzothiazolone and its nitrogen-substituted derivatives were investigated. For this purpose, the effects of the synthesized compounds on violacein production by Chromobacterium violaceum, inhibition and eradication of biofilm formed by Pseudomonas aeruginosa, inhibition of proteolytic activity, and their influence on bacterial swimming and swarming motilities were examined. Among the synthesized compounds, benzothiazolone (Q1) and N-acetylbenzothiazolone (Q8) were determined to be the most active QS inhibitors, particularly with their effects on pigment and biofilm inhibition. Benzothiazolone derivative QS inhibitor compounds may serve as lead compounds for future studies.

Keywords: *Antibacterial activity, benzothiazolone, quorum sensing inhibitors.*

Bazı Benzotiyazolon Türevlerinin Sentezi ve Bunların Quorum Sensing Üzerindeki Etkileri

ÖZ

Bakterilerin popülasyon yoğunluklarını algılayarak iletişim kurmalarını ve özellikle patojenik bakterilerde biyofilm oluşumu ve virülans faktörü üretimini düzenlemelerini sağlayan çoğunluk algılama (Quorum Sensing, QS), bakteriyel enfeksiyonların kontrolü için yeni bir hedef olarak ortaya çıkmıştır. Bu çalışmada, benzotiyazolon ve azot-sübstitüe türevlerinin QS inhibitör etkileri araştırılmıştır. Bu amaçla, sentezlenen bileşiklerin Chromobacterium violaceum'un violacein üretimi üzerine etkileri, Pseudomonas aeruginosa tarafından oluşturulan biyofilmin inhibisyonu, biyofilm eradikasyonu, proteolitik aktivitenin inhibisyonu, bakterinin yüzme ve sürünme hareketleri üzerindeki etkileri araştırılmıştır. Sentezlenen bileşikler arasında benzotiyazolon (Q1) ve N-asetilbenzotiyazolon (Q8), özellikle pigment ve biyofilm inhibisyonu üzerindeki etkileriyle en aktif QS inhibitörleri olarak belirlenmiştir. Bulunan benzotiyazolon türevi QS inhibitörü bileşikler gelecekteki çalışmalarda öncü bileşik olabilir.

Anahtar Kelimeler: *Antibakteriyel aktivite, benzotiyazolon, çoğunluk algılama inhibitörleri.*

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[*] ORCID: 0000-0002-8250-7096, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06330, Ankara, Türkiye
[**] ORCID: 0000-0002-5007-5209, Department of Biology, Faculty of Science, Gazi University, 06500, Ankara, Türkiye
[***] ORCID: 0000-0002-5147-7015, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06330, Ankara, Türkiye
[****] ORCID: 0000-0002-5292-9554, Department of Medical Services and Techniques, Vocational School of Health Services, Gazi University, 06830, Ankara, Türkiye

° Corresponding Author; Yasemin Dündar
E-mail: yasemina@gazi.edu.tr

INTRODUCTION

Quorum sensing (QS) is a bacterial communication mechanism that regulates biofilm formation, virulence factors, bioluminescence, and swarming. In the QS system, autoinducers (AIs) released by bacteria activate target genes when the bacterial population increases. Gram-negative bacteria mostly use *N*-acylhomoserine lactones (AHLs), while Gram-positive bacteria communicate via autoinducer peptides (AIPs). There are also QS compounds used for inter-species communication (Maha Swetha et al., 2024; Vashistha et al., 2023; Wu & Luo 2021; Zhang et al., 2024).

Pseudomonas aeruginosa (*P. aeruginosa*), a multidrug-resistant Gram-negative bacterium, has three major QS signal molecules: *N*-3-oxododecanoyl-L-homoserine lactone (**1**, the Las system), *N*-butyryl-L-homoserine lactone (**2**, the Rhl system), and 2-heptyl-3-hydroxy-4-quinolone (**3**, the *Pseudomonas* quinolone signal system) (Curran et al., 2018; Papenfort & Bassler 2016; Yashkin et al., 2021) (Figure 1.).

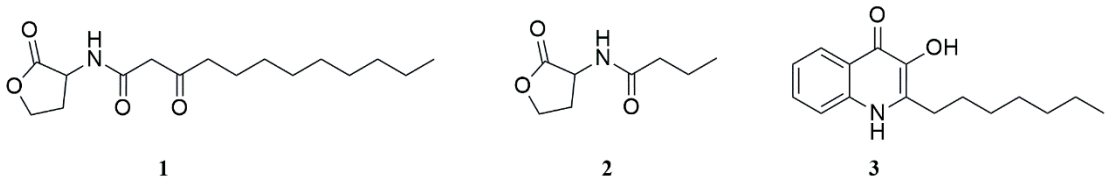


Figure 1. QS signal molecules of *Pseudomonas aeruginosa* (1-3)

Recent studies have shown that inhibition of the QS system can effectively regulate bacterial populations and suppress the expression of virulence factors. This strategy presents a novel and promising avenue for the development of antibacterial agents. Over the past two decades, a wide variety of QS inhibitors have been developed to combat bacterial infections. Notably, most research has focused on Gram-negative bacteria, especially *P. aeruginosa*, and targeting acyl-homoserine lactone (AHL)-mediated signaling pathways and AHL-related furanones as well as their bioisosteric heterocyclic compounds (**4-13**) (Boursier et al., 2019; Estephane et al., 2008; Goh et al., 2015; Jiang et al., 2020; Li et al., 2018a; Lizarme-Salas et al., 2021; Maiga et al., 2025; Yan et al., 2024; Zhang et al., 2023) (Figure 2.).

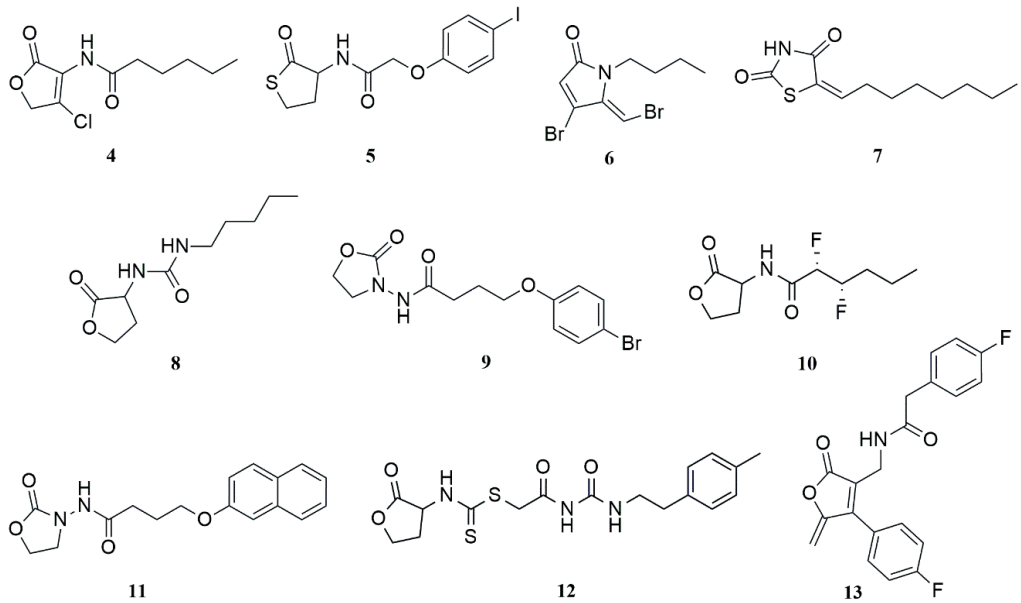


Figure 2. Examples of QS inhibitors

In our previous studies, some *N*-acylbenzoxazolone (**14**) and *N*-acylbenzimidazolone (**15**) derivatives were identified as QS inhibitors. In particular, core rings and short-chain acyl derivatives exhibited QS inhibitory activity (Cevizci et al., 2015; Miandji et al., 2012; Önem et al., 2018). As a continuation of this research, benzothiazolone, which is the bioisostere of benzoxazolone and benzimidazolone heterocyclic compounds, was selected. In the present study, the synthesis of benzothiazolone and *N*-substituted benzothiazolone derivatives (**Q1–Q10**) and their QS inhibitory activities are presented (Figure 3.).

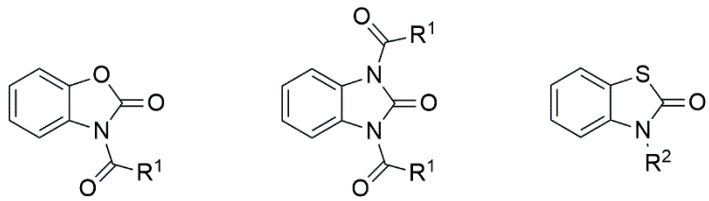


Figure 3. General structures of *N*-acylbenzoxazolones, *N*-acylbenzimidazolones, and *N*-substituted benzothiazolones. R^1: alkyl; R^2: H, NH_2, alkyl, acyl

MATERIALS AND METHODS

Chemistry

All reagents and solvents were obtained from commercial suppliers and used without further purification. Melting points were determined using a Schmelzpunkt SMP-II Digital Melting Point Apparatus and are reported uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker Avance Neo 500 MHz High-Performance Digital FT-NMR spectrometer at the Gazi University Basic and Engineering Sciences Central Laboratory Application and Research Center (GUTMAM). HRMS spectra were obtained using a Waters LCT Premier XE orthogonal acceleration time-of-flight (oa-TOF) mass spectrometer with ESI(+) ionization method. FT-IR spectra were acquired using a Perkin Elmer Spectrum 400 FTIR-ATR spectrometer. Compounds Q1, Q2, Q4, Q5, Q7-Q10 were previously reported (Ashurov et al., 2018; Carato et al., 2004; Dündar et al., 2007; Erdogan et al., 2021; Guenadil et al., 2011; Önkol et al., 2012).

3-Amino-1,3-benzothiazol-2(3*H*)-one (Q3). 6 mmol 1,3-benzothiazol-2(3*H*)-one was dissolved in 5 mL of ethanol, and 0.6 g of NaOH solution in 5 mL of water was added. Then, 6 mmol hydroxylamine-O-sulfonic acid was added portionwise to the solution. After stirring for 3 h, the resulting precipitate was filtered, washed with water, and dried. The product was obtained in 0.35 g yield (35%). Mp: 133-135 °C. HRMS calc. for $C_7H_6N_2OS$ [M+H]; 167.0279, found: 167.0279. ^{1}H-NMR (500 MHz, DMSO-d_6) δ = 7.61 (1H, d, *J*=7.5 Hz, Ar-H7), 7.32-7.40 (2H, m, Ar-H4,5), 7.18 (1H, t, *J*=7.5 Hz, Ar-H6), 5.62 (2H, s, NH_2) ppm. ^{13}C-NMR (125 MHz, DMSO-d_6) δ = 111.9 (Ar-C), 118.7 (Ar-C), 123.2 (Ar-C), 123.4 (Ar-C), 127.0 (Ar-C), 138.6 (Ar-C), 168.5 (C=O) ppm. FT-IR: ν̃ = 3318-3191 (NH_2), 1624 (C=O) cm^{-1}.

3-Butanoyl-1,3-benzothiazol-2(3*H*)-one (Q6). 6 mmol 1,3-benzothiazol-2(3*H*)-one was dissolved in 25 mL of acetone, and a solution of 0.4 g of NaOH in 3 mL of water was added. Then, 7.5 mmol butanoyl chloride was added to the solution and stirred at room temperature for 30 min. At the end of the reaction, water was added to the reaction, and the resulting precipitate was filtered, washed with water, dried, and crystallized with ethanol. The product was obtained in 0.70 g yield (52%). Mp: 91 °C. HRMS calc. for $C_{11}H_{11}NO_2S$ [M+H]; 222.0589, found: 222.0595. ^{1}H-NMR (500 MHz, DMSO-d_6) δ = 8.17 (1H, d, *J*=8.4 Hz, Ar-H4), 7.69 (1H, d, *J*=7.7 Hz, Ar-H7), 7.39 (1H, t, *J*=7.8 Hz, Ar-H5), 7.32 (1H, t, *J*=7.5 Hz, Ar-H6), 3.04 (2H, t, *J*=7.3 Hz, -CH_2), 1.64-1.71 (2H, m, -CH_2), 0.96 (3H, t, *J*=7.4 Hz, -CH_3) ppm. ^{13}C-NMR (125 MHz, DMSO-d_6)

δ = 13.8 (CH_3), 17.7 (CH_2), 40.5 (CH_2), 117.5 (Ar-C), 121.8 (Ar-C), 123.1 (Ar-C), 125.8 (Ar-C), 127.4 (Ar-C), 135.1 (Ar-C), 170.9 (C=O), 174.3 (C=O) ppm. FT-IR: ῡ = 2964 (aliphatic CH), 1697 (C=O), 1683 (C=O) cm^{-1}.

Biological activity

Bacterial strains and culture media

The microorganisms used in this study included *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *methicillin-resistant Staphylococcus aureus* (MRSA) ATCC 43300, *vancomycin-resistant Enterococcus faecalis* (*E. coli faecalis*) (VRE) ATCC 51299, *Escherichia coli* ATCC 25922, Klebsiella pneumoniae (*K. pneumonia*) ATCC 13883, *P. aeruginosa* PAO1, and *Chromobacterium violaceum* (*C. violaceum*) CV12472.

Determination of antimicrobial activity (minimum inhibitory concentration)

Antimicrobial activity of the chemicals was determined by the broth microdilution method against the tested strains according to the Clinical Laboratory and Standards Institute Guidelines (CLSI, 2020). Serial dilutions of the chemicals, prepared from DMSO stock solutions, were made in Mueller-Hinton broth at concentrations ranging from 0.5 to 64 µM in 96-well microtiter plates. Azithromycin (Sigma) was used as a positive antibiotic control. Microbial inocula were prepared from a 24 h fresh culture, suspensions in physiological saline solution were adjusted to 0.5 McFarland standard turbidity ($1.5x10^8$ cfu mL^{-1}), and were diluted 10^{-1} in physiological saline solution. 5 µL of the diluted microbial suspension was added to each well. Plates were then incubated for 24 h at 37 °C. After incubation, the minimum inhibitory concentration (MIC) values of the chemicals were determined by the absence of visible turbidity and confirmed by subculturing 10 µL from non-turbid wells onto an appropriate growth medium. All assays were performed at least three times, and the mean MIC values were selected (Aydın, 2021).

Anti-quorum sensing activity

Screening for violacein production

Agar well diffusion assay was performed with *C. violaceum* ATCC 12472 for the determination of pigment inhibition activity of the synthesized compounds. The bacterial strain was incubated in LB broth at 30 °C for 24 h, and 1% active culture was added to warm LB soft agar (LB Broth containing 0.5% agar) and poured onto LB hard agar. After solidification, 6 mm diameter wells were created, and 50 µL of each chemical at the appropriate sub-MIC concentration was added. Plates were incubated at 30 °C for 48 h, and the diameter of the zone of violacein pigment inhibition was measured and compared with the control groups. The solvent was used as the negative control, and azithromycin served as the positive control (Patel et al., 2022).

Screening for biofilm inhibition and eradication

The anti-biofilm activity of the synthesized compounds against *P. aeruginosa* PAO1 was evaluated using the crystal violet staining assay. Briefly, 10 µL of overnight bacterial growth (adjusted to 0.5 McFarland standard) was added to LB broth supplemented with 1% (w/v) glucose, containing sub-MIC concentrations of the test compounds in a dose-dependent manner. The final volume in each well of a 96-well microtiter plate was 200 µL. Plates were incubated at 37 °C for 24 h to allow biofilm formation. Following incubation, planktonic cells were carefully removed by washing the wells three times with sterile distilled water. The remaining surface-adherent biofilm was fixed with 200 µL of methanol for 15 min. After discarding the methanol and air-drying the wells, the biofilms were stained with 0.1% (w/v) crystal violet solution for 15 min. Excess stain was removed by washing with sterile distilled water, and the bound dye was then solubilized with 95% ethanol. The absorbance was measured at 570 nm using an ELISA plate reader to quantify biofilm biomass. Each assay was performed in quadruplicate. Wells containing only bacterial culture and medium served as the growth control (Venkatramanan et al., 2020).

The percentage of biofilm inhibition was calculated using the following formula:

Biofilm inhibition (%) = [(OD growth control – OD sample) / OD growth control] × 100.

The biofilm eradication assay was performed with slight modifications to the biofilm inhibition method described above. Biofilms were first allowed to form in 96-well microtiter plates as described in the biofilm inhibition method. After this initial incubation period, the contents of the wells were carefully discarded. Aliquots of the prepared chemical solutions were added to the microtiter plate wells together with LB broth to give final sub-MIC concentrations per 200 µl well volume. Sterile LB broth was used as a negative control at a 200 µl well volume. The plates were then incubated at 37 °C for 24 h. Following the second incubation period, the plates were prepared for absorbance measurement as described in the biofilm inhibition method (Hamid, 2019). The results were expressed as percentage inhibition using the following equation:

% Inhibition = [(Absorbance(sample) – Absorbance(control))/ Absorbance(control)] x 100

Inhibition of proteolytic activity

The effect of chemicals on the proteolytic activity of the *P. aeruginosa* PAO1 was evaluated using the well-diffusion method on skim milk agar. Agar plates were punched with 6 mm diameter wells and filled with 50 µl of 0.5 McFarland standard turbidity ($1.5x10^8$ cfu mL^{-1}) of *P. aeruginosa* PAO1 strain and sub-MIC concentrations of the chemicals. Plates were incubated at 37 °C for 24 h and 48 h. Distilled water was used as the negative control. Protease inhibitory activity was estimated by measuring the clear zone around the wells (Kumar et al., 2022; Sırıken et al., 2021). The percentage of protease inhibition was calculated using the following formula: [(Zone diameter of control – Zone diameter of sample) / Zone diameter of control] × 100.

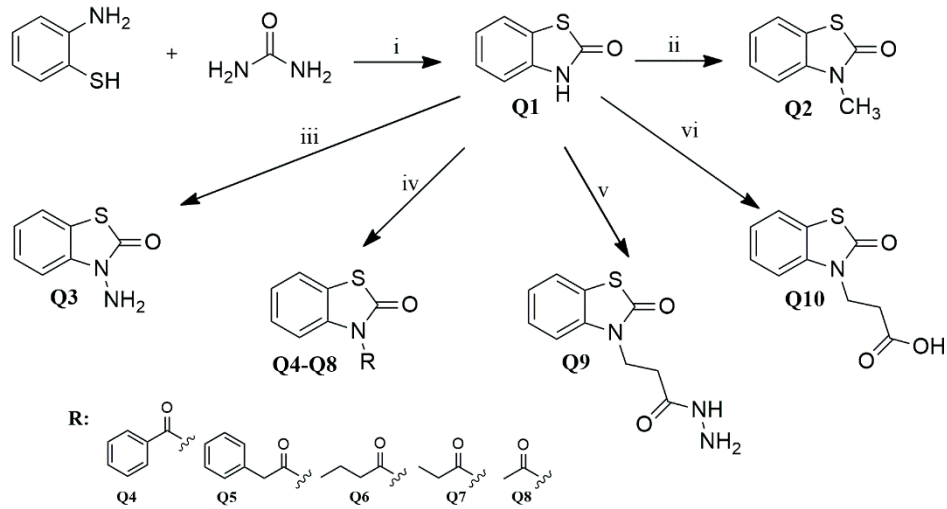
Inhibition of swimming and swarming motility

Swimming and swarming motility inhibition assays were conducted using a slightly modified version of the method described by Li et al. (2018b). Briefly, 5 µL of an overnight culture of *P. aeruginosa* PAO1 ($1.5x10^8$ cfu mL^{-1}) was inoculated at the center of swimming (1% tryptone, 0.5% NaCl, and 0.3% agar) and swarming (1% peptone, 0.5% NaCl, 0.5% agar, and 0.5% filter-sterilized d-glucose) plates containing sub-MIC concentrations of the chemicals. Plates were incubated at 30 °C for 48 h, and the diameters of the motility zones were measured. The percentage reduction in swimming and swarming motility was calculated using the following formula: [(Zone diameter of control – Zone diameter of sample) / Zone diameter of control] × 100.

RESULTS AND DISCUSSION

Chemistry

The benzothiazolone core (Q1) was synthesized via the condensation of urea and 2-aminothiophenol. The *N*-methyl derivative (Q2) was prepared by reaction with dimethyl sulfate (DMS), and the *N*-NH_2 derivative (Q3) was obtained by reaction with hydroxylamine-O-sulfonic acid (HOSA). *N*-acyl derivatives (Q4-Q8) were synthesized by treating benzothiazolone with the corresponding acyl halides in aqueous sodium hydroxide. For the propanhydrazide (Q9) and propanoic acid (Q10) derivatives, the methyl propanoate intermediate was first prepared, followed by Michael addition with benzothiazolone and methyl acrylate. Subsequently, Q9 was obtained via reaction of the ester with hydrazine hydrate, while Q10 was obtained through ester hydrolysis using concentrated HCl (Scheme 1.).



Scheme 1. Synthesis of compounds Q1-Q10. Reagents and conditions. (i) 180 °C; (ii) DMS, NaOH, water; (iii) HOSA, NaOH, water/ethanol. (iv) Appropriate acyl halide, NaOH, water/acetone; (v) a. methyl acrylate, TEA b. $NH_2NH_2.H_2O$, ethanol; (vi) a. methyl acrylate, TEA, b. const. HCl.

Biological activity

Inhibiting intercellular bacterial communication and preventing the production of virulence factors or biofilm formation represents an alternative therapeutic strategy to combat bacterial infections caused by antibiotic-resistant bacterial strains. In this study, we evaluated the anti-virulence potential of a series of synthesized benzothiazolone derivatives against *C. violaceum* ATCC 12472 and *P. aeruginosa* PAO1, two well-characterized Gram-negative pathogens with QS-regulated virulence phenotypes.

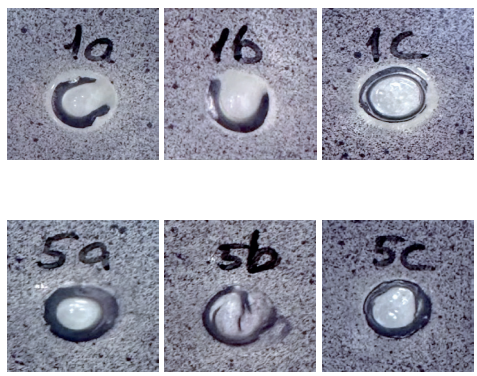
First of all, the antibacterial activities of the synthesized compounds were evaluated against a panel of bacterial strains, including *S. aureus* ATCC 25923, *methicillin-resistant S. aureus* (MRSA) ATCC 43300, *vancomycin-resistant E. faecalis* (VRE) ATCC 51299, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *P. aeruginosa* PAO1, and *C. violaceum* CV12472. MIC assays revealed that the compounds demonstrated weak antimicrobial activity against the tested strains. The most active compound against *C. violaceum* ATCC 12472 was benzothiazolone (Q1), with an MIC value of 1 mM, whereas Q1, its *N*-methyl derivative (Q2), and *N*-acetyl derivative (Q8) were effective against *P. aeruginosa* PAO1 at an MIC value of 4 mM (Table 1.).

An agar well diffusion assay was performed using *C. violaceum* ATCC 12472 to evaluate the anti-QS activity of the synthesized compounds through pigment inhibition. The QS inhibition assay revealed that most of the tested compounds reduced violacein pigment production at sub-MIC concentrations, supporting their potential as quorum sensing inhibitors (QSIs). Among them, Q1 was the most potent, showing pronounced QS inhibition at 0.25 mM concentration. Moreover, Q8 and *N*-propanoic acid derivative (Q10) demonstrated effective inhibition at ≥0.5 mM. In contrast, the *N*-phenacetly derivative (Q5) was inactive at sub-MIC concentrations ≤3 mM (Table 1. and Figure 4.).

Table 1. Minimum Inhibitory Concentration (MIC) activity and pigment inhibition of the synthesized compounds

Comp.	A	B	C	D	E	F	G	Pigment inhibition
Q1	8 mM	16mM	16mM	4mM	8 mM	4mM	1 mM	≥0.25 mM
Q2	>4 mM	>4mM	>4mM	4mM	>4mM	4mM	2 mM	≥1 mM
Q3	16mM	>16mM	>16mM	>16mM	>16mM	16mM	4 mM	≥1 mM
Q4	16mM	>16mM	>16mM	>16mM	16mM	16mM	8 mM	≥4 mM
Q5	4mM	8mM	8mM	4mM	16mM	8mM	4 mM	-
Q6	>16mM	>16mM	>16mM	>16mM	>16mM	16mM	8 mM	≥4 mM
Q7	4mM	8mM	4mM	8mM	<8mM	8mM	4 mM	≥3 mM
Q8	16mM	16mM	>16mM	8mM	16mM	4mM	2 mM	≥0.5 mM
Q9	>32mM	>32mM	>32mM	>32mM	>32mM	32mM	8 mM	≥7 mM
Q10	2mM	8mM	8mM	4mM	8mM	8mM	2 mM	≥0.5 mM

A: *S. aureus* ATCC 25923, B: *Methicillin-resistant S. aureus* (MRSA) ATCC 43300, C: *Vancomycin-resistant E. faecalis* (VRE) ATCC 51299, D: *E. coli* ATCC 25922, E: *K. pneumoniae* ATCC 13883; F: *P. aeruginosa* PAO1, G: *C. violaceum* CV12472.



Q1a- 0.75 mM (+)
Q1b- 0.50 mM (+)
Q1c- 0.25 mM (+)

Q5a- 3 mM (-)
Q5b- 2 mM (-)
Q5c- 1 mM (-)

Figure 4. Representative examples of the pigment inhibition assay.

The crystal violet staining assay was used to determine biofilm inhibition and eradication activities of the synthesized compounds against *P. aeruginosa* PAO1. All compounds were tested at three sub-MIC concentrations (Table 2). Among the tested derivatives, Q1, Q2, Q5, Q7, and particularly Q8 exhibited promising biofilm inhibition and eradication activities. Analysis of intra- and inter-group results revealed that Q8 was the most effective in inhibiting biofilm formation, achieving 85.15% inhibition at 1 mM concentration. In the biofilm eradication assay, Q1 and *N*-methyl derivative Q2 displayed activity, eradicating over 80% of pre-formed biofilms at 2 mM. This is particularly noteworthy since the removal of mature biofilms is often more difficult than preventing their formation. These results suggest that the compounds not only interfere with initial biofilm development but may also disrupt established biofilm structures.

The effects of the synthesized compounds on the proteolytic activity of *P. aeruginosa* PAO1 were assessed using the well-diffusion method on skim milk agar. Among the tested compounds, Q2 exhibited 12.80% inhibition at 3 mM, and Q3 showed 10.34% inhibition at 15 mM concentration. The remaining compounds displayed less than 10% inhibition at the sub-MIC concentrations tested.

Table 2. % Biofilm inhibition and eradication of the synthesized compounds at sub-MIC values

Comp.	*P. aeruginosa* PAO1 MIC	Biofilm inhibition/ Biofilm eradication (%) sub-MIC values (mM)		
		3 mM	**2 mM**	**1 mM**
Q1	4mM	91.04±0.54 / 86.89±3.48	69.38±4.51 / 80.78±4.11	19.77±1.03 / ni
Q2	4mM	80.57±3.61 / 83.88±3.26	45.08±2.62 / 81.69±5.67	40.44±2.49 / ni
Q8	4mM	90.97±1.05 / 75.09±7.54	90.79±0.65 / 70.48±2.05	85.15±3.57 / ni
		7 mM	**6 mM**	**4 mM**
Q5	8mM	90.52±0.49 / 81.49±6.22	90.07±0.61 / 78.07±5.59	89.63±0.27 / 70.17±11.8
Q7	8mM	90.52±0.70 / 80.56±3.22	89.66±1.89 / 72.85±2.89	88.08±2.60 / ni
Q10	8mM	83.52±5.29 / 49.13±1.62	87.03±4.63 / 54.11±7.95	ni / ni
		15 mM	**10 mM**	**5 mM**
Q3	16mM	91.50±0.70 / 66.58±5.65	88.98±3.67 / 56.63±7.47	85.36±5.74 / 40.69±5.46
Q4	16mM	90.30±1.53 / ni	89.24±1.93 / ni	ni / ni
Q6	16mM	89.24±0.04 / 52.44±3.35	85.76±3.49 / ni	71.09±0.04 / ni
		30 mM	**20 mM**	**10 mM**
Q9	32mM	87.68±4.96 / 77.28±2.32	86.59±4.43 / 88.98±0.05	88.68±3.24 / 68.60±2.52

The motility assays provided further insight into the anti-virulence profiles of the compounds. Swimming and swarming motility inhibition assays were conducted using *P. aeruginosa* PAO1 (Figure 5). All compounds demonstrated inhibitory effects on both types of motility to varying degrees. Notably, swarming motility was more significantly inhibited (36.70%-67.40%) compared to swimming motility (12.53%-26.76%) across all tested compounds. Among these, Q6 (*N*-butanoyl derivative) exhibited the highest inhibitory activity, with 67.40% swarming and 15.49% swimming inhibition. Similarly, Q5 (*N*-phenylacetyl derivative) showed high swarming inhibition (60.44%). Q1, which was the most active compound in the violacein pigment inhibition assay and also a potent biofilm eradicator, inhibited swarming by over 60%. In addition, Q1 and Q10 inhibited swimming motility by more than 25%.

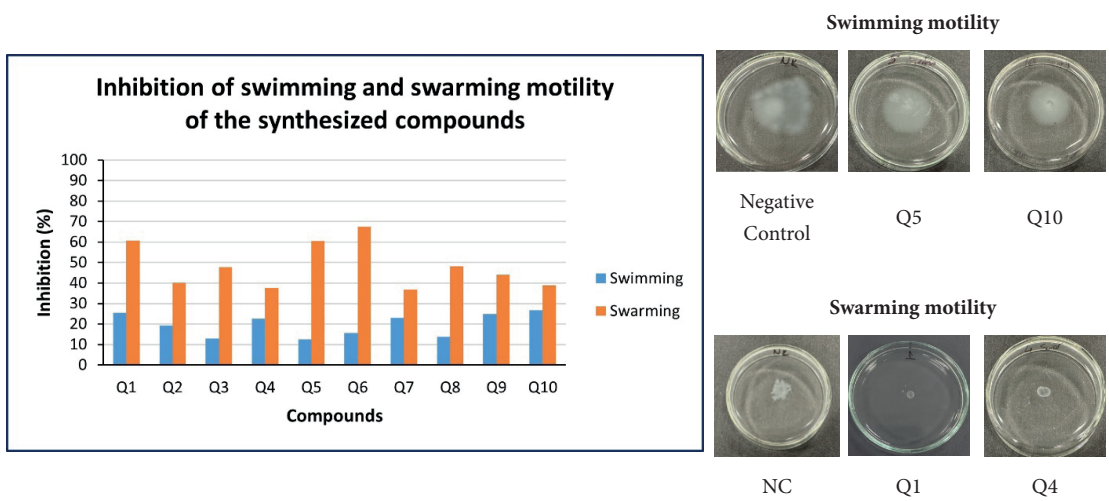


Figure 5. Percentage inhibition of swimming and swarming motility of the synthesized compounds at 1 mM, with representative plate images.

CONCLUSION

In summary, the synthesized benzothiazolone derivatives exhibited promising anti-virulence properties against *C. violaceum* ATCC 12472 and *P. aeruginosa* PAO1, targeting QS, biofilm formation, and motility without exerting strong bactericidal effects. The benzothiazolone core (Q1), *N*-acetyl derivative (Q8), and *N*-propanoic acid derivative (Q10) significantly inhibited violacein production at sub-MIC concentrations, indicating their potential as quorum sensing inhibitors and in agreement with our previous studies (Cevizci et al., 2015; Miandji et al., 2012; Önem et al., 2018). Crystal violet biofilm assays further confirmed the anti-biofilm potential of these compounds: Q8 demonstrated the highest inhibition of biofilm formation (85.15% at 1 mM), while Q1 and *N*-methyl derivative Q2 were particularly effective in eradicating mature biofilms (over 80% at 2 mM). Motility assays showed that all compounds interfered with swimming and swarming behaviors to varying degrees, with swarming motility being more susceptible. Q6 (*N*-butanoyl derivative) exhibited the highest swarming inhibition (67.40%), while Q1 and Q10 also inhibited swimming motility (>25%). These findings demonstrate that several of the synthesized compounds exhibit multi-targeted anti-virulence activity at sub-MIC levels. Thus, benzothiazolones may serve as valuable scaffolds for the development of novel therapeutics that target bacterial communication and virulence, a promising approach to combat antibiotic-resistant infections.

AUTHOR CONTRIBUTION STATEMENT

Design, synthesis and structure elucidation of the title compounds (ÖKÖ and YD), biological activity (MBK and EB). The manuscript preparation and editing (ÖKÖ, YD, MBK, and EB).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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