Original Article

# IN VITRO MICROBIAL TIME-KILLING CURVE FOR NEWLY SYNTHESIZED AMINOACETYLENIC-2-MERCAPTOBENZOTHIAZOLE COMPOUND

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## ABSTRACT

**Objective:** To determine the time needed for killing different types of microorganisms by a newly synthesized 2-mercapto-1,3-benzothiazole derivative in comparison to ciprofloxacin and fluconazole.

**Methods**: The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) for 2-{[4-(2,6-dimethylPiperidin-1-yl)but-2-yn-1-yl]Sulfanyl}-1,3-benzothiazole(AZ3) compound were determined, using the broth dilution method. The MBC and MFC dilutions were prepared. Broth cultures of *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) were incubated at 37 °C for 24 h, and *Candida albicans* (*C. albicans*) was incubated at 25 °C for 48 h. 0.1 ml of each broth culture represent $1.5 \times 10^6$ CFU/ml was challenged with 9.9 ml broth containing the MBC or MFC concentrations of the AZ3 compound. From each sample at different time intervals, 1 ml was taken and added to 9 ml of sterile distilled water, in order to neutralize the effect of AZ3. Serial dilution was done and a viable count was determined from the appropriate dilutions.

**Results:** The viability of the *P. aeruginosa*, *E. coli*, *S. aureus*, *B. subtilis* and *C. albicans* were killed within 3.5 h, 5 h, 24 h, 3 h and 5 h respectively. The time killing curves showed that AZ3 needed longer time for killing *S. aureus* than the time needed to kill *B. subtilis*. On the other hand, AZ3 needed a shorter time to kill *P. aeruginosa,* than the time needed to kill *E. coli*. In comparison with ciprofloxacin, AZ3 needed a shorter time to kill *P. aeruginosa* and *E. coli*, and the same time to kill *B. subtilis*, while it needed longer time than ciprofloxacin to kill *S. aureus*. In comparison with fluconazole, AZ3 with lower MFC than fluconazole needed longer time to kill *C. albicans*.

**Conclusion:** AZ3 showed promising antimicrobial killing activities, in compared with ciprofloxacin and fluconazole, which promoted our interest to investigate the time of killing needed for other 2-mercaptobenzothiazole derivatives against different types of microorganisms.

**Keywords:** 2-mercaptobenzothiazole derivatives, Alkylation, Mannich reaction, Time-kill curve

## INTRODUCTION

Molecules with benzothiazole (BTA) moiety have different biological activities [1]. Accordingly, 2-mercaptobenzothiazole (2-MBT) derivatives have a variety of applications [2]. 2-MBT was isolated as a natural product, from fermentation cultures of micrococcus species bacterial symbiont of the marine sponge *Tedaniaignis* [3]. 2-MBT consists of benzothiazole which is a heterocyclic compound includes benzene ring fused with 4,5-positions of the thiazole ring [4, 5], and the mercapto (thiol group) substituent at position 2 of thiazole ring that gives the compound antibacterial and anti-inflammatory activity [6]. 2MBT is prepared by reaction between 2-amino-thiophenol with refluxing carbon disulfide [6], in the presence of acetic anhydride [7]. Novel 4-substituted phenyl–3-chloro-1-[(benzothiazolythio) accetamidyl]-2-azetidinone derivatives were synthesized (fig. 1). These novel compounds were screened for their antibacterial and antifungal activities against *S. aureus*, *A. niger*, and *C. albicans*. The study showed that compound derivatives having 3-OH–$C_6H_4$ and Cl-$C_6H_4$ groups, promoted the activity against candida while compounds derivatives having 4-OH-$C_6H_4$ and 2-Cl-$C_6H_4$, at position 4 in the azetidinone nucleus are very effective against *S. aureus* and *A. niger* [8].
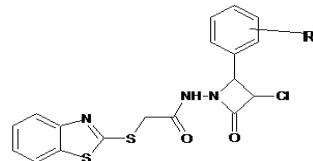


R= 4-$NO_2$/3,4,5-Tri-$OCH_3$/2-OH/3-OH/4-OH/2-$OCH_3$/4-$OCH_3$/2-Cl/3-Cl/4-Cl

**Fig. 1: 4-substituted phenyl–3-chloro-1-[(benzothiazolythio) accetamidyl]-2-azetidinone derivatives [8]**

Kuchta *et al.* in 1989 carried out a research which involved 22 derivatives of 2-alkylthio-6-amino and 2-alkyl thio-6-formamidobenzothiazole [9]. They were tested against 15 candida yeast strains, and against *A. niger* +with a suspension of spore free mycelium homogenate as inoculums. They found that no significant effect was observed against *A. niger*, The pathogenic species *C. albicans* and *C. gnilliemondii*. In another study, 3-(2-alkylsulfanyl-6-benzothiazolylaminomethyl)-2-benzothiazolethiones were synthesized, by the reaction of 2-alkylsulfonyl-6-aminobenzothiazoles with 3-hydroxy–2-benzoxazolethione in ethanol (fig. 2). New derivatives were tested against candida, researchers found that derivatives having the benzyl group at the second position of MBT ring demonstrated maximum inhibition of the oxygen evolution rate in spinach chloroplasts [10].
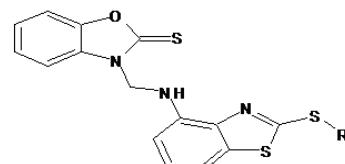


R=$CH_3$/$C_2H_5$/allyl/$(CH_2)CH_3$/$CH(CH_3)C_2H_5$/$(CH_2)_4CH_3$($CH_2)_5CH_3$/($CH_2)_6CH_3$/($CH_2)_7CH_3$/($CH_2)_8CH_3$/$CH_2C_6H_5$

**Fig. 2: 3-(2-alkylsulfanyl-6-benzothiazolylaminomethyl)-2-benzothiazolethiones [9]**

In this study, *in vitro* time exposure needed for killing different types of microorganisms by AZ3 was determined, and were compared to

the time killing curves for ciprofloxacin and fluconazole against the same microorganisms. The main aim of this study is finding new promising 2-mercaptobenzothiazole derivatives, which have the ability to kill different microorganisms in competitive time compared to other drugs in the market.

## MATERIALS AND METHODS

### Experimental

### Chemicals

2-mercaptobenzothiazole 97%, propargyl bromide, cis-2,6-dimethylpiperidine 98%, all of them were purchased from (Sigma Aldrich, USA), magnesium sulphate anhydrous (Lonover, UK), potassium bromide (KBr) (Scharlau, Spain), potassium carbonate anhydrous ($K_2CO_3$) (Gainland Chemical Company (GCC, UK), paraformaldehyde polymer (BDH chemicals Ltd Poole, England), cuprous chloride LRG (east Anglia Chemicals Hadleighlpswich), acetonitrile 99.7% (PanReAcSintesis)/(PanReAcQuimca SA, EU), 1,4-dioxane HPLC/ACS (FULL Time, China), chloroform (stabilized with 0.5-1% ethanol) (TEDIA, USA), dimethyl sulfoxide (DMSO) (BBC Chemicals for lab, EU), diethyl ether (Lonover, England)/(RCL Labs can, Thailand), absolute ethanol 99.9% (Super Chem, USA), acetone 99% (Scharlau, Spain), distilled water (Ultra, Jordan).

### Instrumentation

analytical balance with a precision 0.01 mg (Phoenix instrument, USA), hot plate with magnetic stirrer (Dragon, China), rotary evaporator 0-100Kpa/0-700 mmHg (Rocker 600, Germany), Buchner funnel pump (Vacuubrand, Germany), melting point apparatus (Gallenkamp, USA), FT-IR spectrophotometer 7800 to 400 cm-1 (Evisa, Poland), DSC (Mettler Toledo, Int Co), UV-VIS (Evolution 160, USA), HPLC-UV (Finnigan Surveyor, USA), NMR 300 MHZ (Varian 300 MHZ, USA), NMR 500 MHZ (Varian 500 MHZ, USA), elemental analyzer with variation range (±4) (Euro Vector, Italy), autoclave machine (Rypa, Spain), incubator (EuroStar, EU), vortex mixer (Labinco, India), hot plate magnetic stirrer (Dragon, China), sterile tubes, sterile swabs (mWe, UK), micropipette (Oxford, USA).

### Synthesis

### Synthesis of 2-(prop-2-yn-1-ylsulfanyl)-1,3-benzothiazole (AZ0)

After the mixture of 2-MBT (5.01 g,0.03 mol), potassium carbonate anhydrous (3 g, 0.03 mol) and 20-40 ml acetonitrile (ACN) has been heated and stirred under reflux for 30 min, the propargyl bromide (5 ml, 0.03 mol) was added drop wise. The reaction mixture was heated and stirred under reflux for 2 h. Then the mixture was filtrated and concentrated under reduced pressure to give a brown syrup. The crude product was extracted with 50 ml chloroform and 50 ml distilled water; chloroform layers were collected, dried over magnesium sulfate and evaporated under reduced pressure. The solid brownish crystals were weighed and characterized through DSC, UV-HPLC, FT-IR, ¹H-NMR (fig. 3).
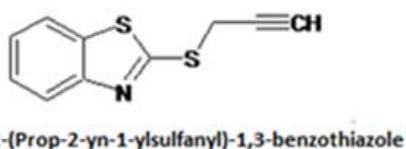


**Fig. 3: 2-(prop-2-yn-1-ylsulfanyl)-1,3-benzothiazole (AZ0)**

### Synthesis of 2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl] sulfanyl}-1,3-

### benzothiazole (AZ3)

A mixture of 2-(prop-2-yn-1-yl sulfanyl)-1,3-benzothiazole (AZ0) (2.062 g, 0.01 mol), paraformaldehyde (0.5 g in excess), cyclic amine (2,6-dimethyl piperidine) and a catalytic amount of cuprous chloride

in 1,4-dioxane (25 ml) was stirred at room temperature, for 10 min then was heated and stirred under reflux at 70-75 °C for three h. The reaction mixture was filtrated and concentrated under reduced pressure, to give a brown syrup which was dissolved in diethyl ether, filtrated and concentrated under reduced pressure. The final products was 2-{[4-(2,6-dimethylpiperidin-1-yl]but-2-yn-1-yl] sulfanyl}-1,3-benzothiazole (AZ3). The title compound AZ3 was synthesized by Mannich reaction in 2.5 g, yield 76.22%, mp: 48.62 °C, UV-HPLC retention time: 2.167 min, FT-IR (KBr cm-1): C-H stretching aromatic (3080.477 cm-1, 2923.556 cm-1), C-H stretching tertiary amine (2850.274 cm-1, 2788.563 cm-1, C=C stretching aromatic (1673.909 cm-1), C=N stretching thiazole (1590.985 cm-1, 1432.851 cm-1), C-N stretching 3 ° aromatic (1373.168 cm-1), C-N stretching tertiary cyclic amine (12226.433 cm-1), C-H out of plane bending aromatic (865.882 cm-1), C-S stretching (717.39 cm-1, 624.823 cm-1), 1H-NMR (DMSO d6): δ; four types of cyclic amine protons: 0.75 ppm (doublet, type A, 6H), 0.95 ppm (sextet, type B, 2H), 1.2–1.35 ppm (quartet, type C, 4H), 2 ppm (quintet, type D, 2H), 3.4 ppm (singlet, C-CH2-N, 2H), 4.2 ppm (singlet, S-CH2C, 2H), four types of aromatic protons: 7.35 ppm (triplet, type A, 2H), 7.45 ppm (triplet, type B, 2H), 7.85 ppm (doublet, type C, 1H), 8 ppm (doublet, type D, 1H).
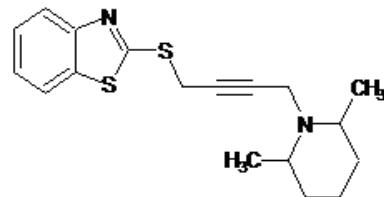


**Fig. 4: Synthesis of 2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole (AZ3)**

### Culture media

Muller hinton agar (MHA) (Mastgrp Ltd, UK)/(Himedia, India), muller hinton broth (MHB) (Mastgrp Ltd, UK), sabourauds dextrose agar (SDA) (Mastgrp Ltd, UK), sabourauds dextrose broth (SDB) (Himedia, India), dimethyl sulfoxide (DMSO) (BBC Chemicals for lab, EU), distilled water (Ultra, Jordan).

### Microorganisms

*Staphylococcus aureus* (*S. aureus* ATCC 6538), *Bacillus subtilis* (*B. subtilis* ATCC 6633), *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC 9027), *Escherichia coli* (*E. coli* ATCC 8739), *Candida albicans* (*C. albicans* ATCC 10231). All these bacterial strains were obtained from Dar Al Dawa (Na'ur, Jordan).

### Determination of microbial time kill curves for AZ3

The minimum inhibitory concentration (MIC) and MBC for AZ3 were determined firstly. The synthesized compound (AZ3) was diluted out in the series of twofold dilutions, in a liquid growth medium dispensed in test tubes [11, 12]. The AZ3 stock solution has to be at least 1000 μg/ml [12]. Accordingly, AZ3 stoke solution was prepared in 1000 μg/ml in 10 ml DMSO, this solution was diluted serially in MHB for bacteria and SDB for fungi, to give concentrations ranging from 500-7.81 μg/ml. The final volume of the compound dilutions in each tube was 5 ml. To these tubes, 0.1 ml of the overnight culture was added. Positive control tube contained 5 ml of sterile broth and 0.1 ml of microbial culture, while the negative control tube contained 5 ml of the tested compound dilution in sterile broth.

The tubes were incubated at 37 °C for 24 h for bacteria, and at 25 °C for 48 h for fungi. The MIC was determined by comparison the turbidity of each concentration tube with the turbidity of the positive control tube; MIC tube is the lowest concentration of the compound in which no turbidity was observed [13]. To determine MBC and MFC, the MIC tube and the tubes with dilutions preceded were sub-cultured onto agar plates, the plates were incubated at 37 °C for 24 h for bacteria, and at 25 °C for 48 h for fungi. The lowest concentration tube that gave no growth was the MBC or MFC. Broth

dilution test was designed to allow for statistical analysis and was performed in triplicates.

Time exposure viability test for AZ3 was carried out, to assess the killing ability rate of AZ3 within a given contact time. The MBC and MFC concentrations were prepared for compound AZ3 using sterile broth. The microbial cultures were prepared and incubated at 37 °C for 24 h for bacteria and at 25 °C for 48 h for fungi. 0.1 ml of each culture with definite inoculum sizes were challenged with 9.9 ml of MBC/MFC concentrations for the AZ3 compound.

Then 1 ml was taken from the sample, at different time intervals as shown in (table 1), and added to 9 ml of sterile distilled water, to neutralize the AZ3 activity (this step was enough to neutralize as primarily detected). Serial dilution using sterile distilled water was done, and the viable count was determined from the appropriate dilutions.

**Table 1: Time intervals used for bacterial killing curve**

| Microorganism | Time intervals (h) | | | | | |
|---|---|---|---|---|---|---|
| S. aureus | 1.5 | 2.5 | 3.5 | 4.5 | 20 | 24 |
| B. subtilis | 0.5 | 1 | 2 | 3 | 4 | 5 |
| E. coli | 1 | 1.5 | 2 | 5 | - | - |
| P. aeruginosa | 1.5 | 2.5 | 3.5 | 4.5 | 5.5 | - |
| C. albicans | 0.5 | 1 | 2 | 2.5 | 3 | 5 |

## RESULTS

### Minimum inhibitory concentrations results

The newly synthesized compound AZ3 showed activity against all types of microorganisms tested (table 2), the minimum inhibitory concentrations (MIC), and minimum bactericidal/fungicidal concentrations (MBC/MFC) were measured (table 2).

### Microbial time-killing curve results

The time exposure viability curve for the addition of 0.1 ml *P. aeruginosa* ATCC 9027 culture, with a viable count of (2.47 X $10^7$) to AZ3 at 250 µg/ml dilution was demonstrated in fig. 5. The 5 log cycle reductions were detected, the curve shows that the bacterial count decreased rapidly over 1 h. Then over 1.5 h, the count of bacteria decreased slowly. The viability of the *P. aeruginosa* abolished within 3.5 h. accordingly, the LT 90 % value (time at which 90% of the original population were killed by the antimicrobial agent) for this experiment was approximately in the period between 2.5 and 3.5 h. In the time-killing test for ciprofloxacin against *P. aeruginosa*, the 7 log cycle reductions were detected, the curve shows that the count of bacteria decreased rapidly within the first 0.5 h, then the count decreased slowly within the 4 h. The viability of the *P. aeruginosa* was abolished within 5 h.

**Table 2: MIC and MBC of AZ3**

| Microorganisms | P. aeruginosa | E. coli | S. aureus | B. subtilis | C. albicans |
|---|---|---|---|---|---|
| MIC(µg/ml) | 125 | 250 | 125 | 62.5 | 125 |
| MBC (µg/ml) | 250 | 500 | 500 | 125 | 250 |

Values are the mean (n=3), AZ3: 2-{[4-(2, 6-dimethylpiperidin-1-yl) but-2-yn-1-yl] sulfanyl}-1,3-benzothiazole.

**Table 3: Bacterial time-killing of AZ3 against *P. aeruginosa* ATCC 9027**

| Microorganism | P. aeruginosa | | | | | |
|---|---|---|---|---|---|---|
| Time (h) | 0 | 1.5 | 2.5 | 3.5 | 4.5 | 5.5 |
| Count (CFU/ml) | 2.47 X$10^7$ | 6.8x $10^3$ | 1.4x $10^3$ | 0 | 0 | 0 |
| Log (CFU/ml)* | 7.39±0.61 | 3.83±0.17 | 3.15±0.85 | 0 | 0 | 0 |

*Values are the mean±SD (n=3), SD: standard deviation, CFU: colony forming unit, AZ3: 2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole.
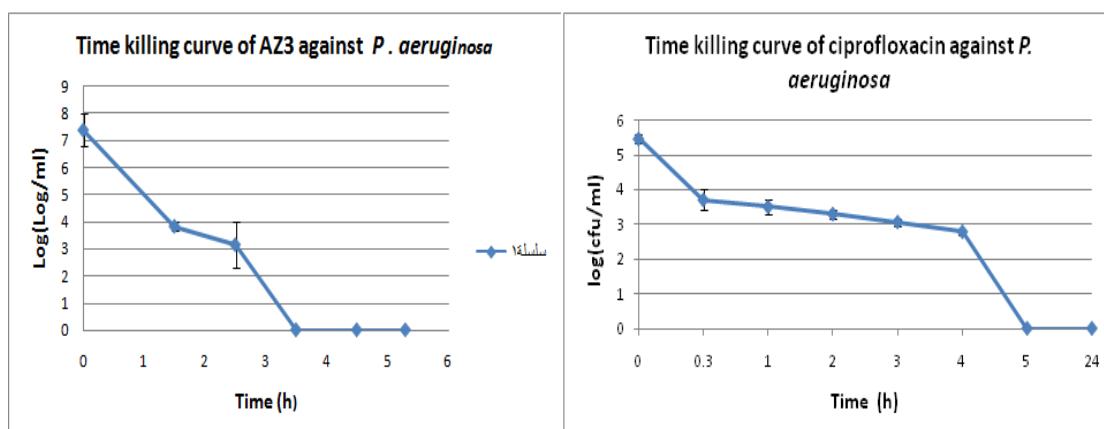


**Fig. 5: Time exposure viability curve for addition of compound AZ3 and ciprofloxacin, at MBC (250 µg/ml) to culture of *P. aeruginosa* ATCC 9027**

Values are the mean±SD (n=3), AZ3: 2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole, CFU: colony forming unit, time (X axis): time intervals needed for killing *P. aeruginosa*, Log (CFU/ml) (Y axis): viability of *P. aeruginosa* at different times.

The time exposure viability curve for the addition of 0.1 ml of *E. coli* ATCC 8739 culture, with a viable count of (1.42 X10^{7}) to AZ3 at 500 µg/ml dilution was demonstrated in fig. 6. The 4 log cycle reductions were obtained in approximately 5h. The count of bacteria decreased

quickly over 1h, then the count of bacteria decreased slowly over the next 1h. The viability of *E. coli* was abolished within 5 h. accordingly, the LT 90% value for this experiment was approximately in the period between 2-5 h.

In The time-killing test for ciprofloxacin against *E. coli*, The 5 log cycle reductions were detected. The curve shows that the count of bacteria decreased within the first 0.5 h, then the count decreased slowly within the 4 h. The viability of *E. coli* was abolished within 5 h.

**Table 4: Bacterial time-killing of AZ3 against *E. coli* 8739**

| Microorganism | *E. coli* | | | | |
|---|---|---|---|---|---|
| Time (h) | 0 | 1 | 1.5 | 2 | 5 |
| Count (CFU/ml) | 1.42 *10[7] | 3.4x10^{3} | 1.63x10^{3} | 3.0x10^{2} | 0 |
| Log (CFU/ml)* | 7.15±0.85 | 3.53±0.47 | 3.21±0.79 | 2.48±0.52 | 0 |

*Values are the mean±SD (n=3), SD: standard deviation, CFU: colony forming unit, AZ3: 2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole.
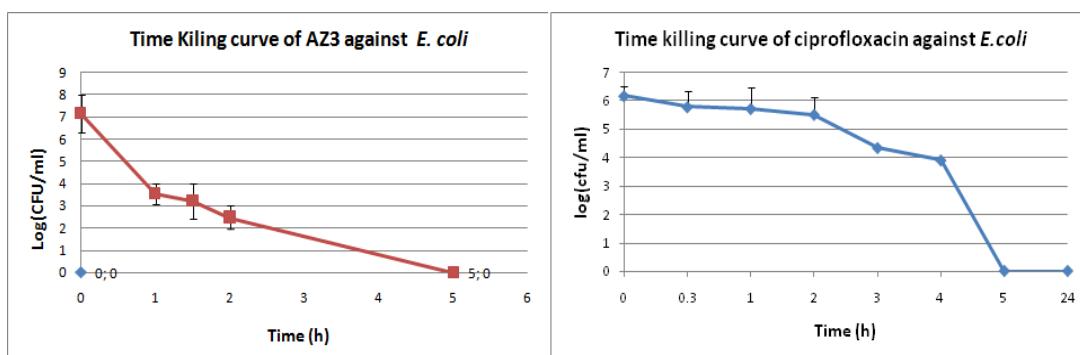


**Fig. 6: Time exposure viability curve for addition of compound AZ3 and ciprofloxacin, at MBC (500 µg/ml) and (125 µg/ml) respectively to culture of *E. coli* ATCC 8739**

Values are the mean±SD (n=3), AZ3: 2-{[4-(2, 6-dimethylpiperidin-1-yl) but-2-yn-1-yl] sulfanyl}-1,3-benzothiazole, CFU: colony forming unit, time (X-axis): time intervals needed for killing *E. coli*, Log (CFU/ml) (Y-axis): viability of microorganisms at different times.

The time exposure viability curve for the addition of 0.1 ml of *S. aureus* ATCC 6538 culture, with a viable count of (1.76x10^{8}) to 500 µg/mlAZ3 dilution was demonstrated in fig. 7. The 6 log cycle reductions were obtained in approximately 24 h. The count of bacteria decreased quickly after 1.5 h, then the count of bacteria

decreased slowly over 18.7 h. The viability of *S. aureus* was abolished within 24 h. Accordingly, the LT 90% value for this experiment was approximately in the period between 20 h and 24 h. AZ3 needed the longer time of exposure to kill all of the original *S. aureus* population.

In the time-killing test for ciprofloxacin against *S. aureus,* the 7 log cycle reductions were detected, the curve shows that the count of bacteria decreased slowly within the first 2 h, then it decreases rapidly between 2 to 3 h. The viability of *S. aureu* was abolished within 5 h.

**Table 5: Bacterial time-killing of AZ3 against *S. aureus* 6539**

| Microorganism | *S. aureus* | | | | | | |
|---|---|---|---|---|---|---|---|
| Time (h) | Zero | 1.5 | 2.5 | 3.5 | 4.5 | 20 | 24 |
| Count (CFU/ml) | 1.76x10^{8} | 1.34x10^{6} | 1.29x10^{6} | 3.4x10^{5} | 5.9x10^{4} | 2.8x10^{4} | 0 |
| (Log CFU/ml)* | 8.25±0.75 | 6.13±0.87 | 6.11±0.89 | 5.53±0.47 | 4.77±0.23 | 4.45±0.55 | 0 |

*Values are the mean±SD (n=3), SD: standard deviation, CFU: colony forming unit, AZ3: 2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole.
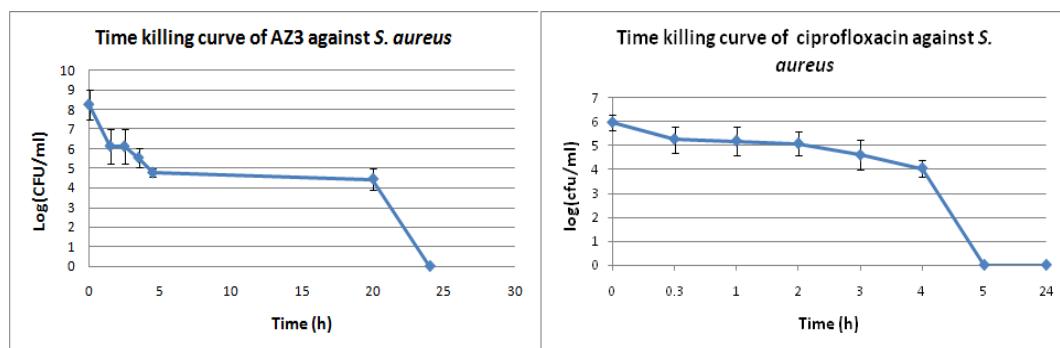


**Fig. 7: Time exposure viability curve for addition of compound AZ3 and ciprofloxacin at MBC (250 µg/ml) to culture of *S. aureus* ATCC 6538**

Values are the mean±SD (n=3), AZ3: 2-{[4-(2,6-dimethyl piperidin-1-yl) but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole, CFU: colony forming unit, time (X-axis): time intervals needed for killing *S. aureus*, Log (CFU/ml) (Y-axis): viability of *S. aureus* at different times.

The time exposure viability curve for the addition of 0.1 ml of *B. subtilis* ATCC 6633 culture, with a viable count 1.08x10³CFU/ml to AZ3 at 125 µg/ml dilution was demonstrated in fig. 8. The 6 log cycle reductions were obtained in approximately 5h. Surprisingly, the

count of bacteria increased over 2 h, then the count of bacteria decreased sharply over the next 1 h. The viability of *B. subtilis* was abolished within 3 h. The LT 90% value for this experiment was approximately in the period between 2 to 3 h. In

The time-killing test for ciprofloxacin against *B. subtilis,* the 7 log cycle reductions were detected, the curve shows that the count of bacteria decreased slowly within the first 2 h. The viability of *B. subtilis* was abolished within 3 h.

**Table 6: Bacterial time-killing of AZ3 against *B. subtilis* 6633**

| Microorganism | *B. subtilis* | | | | | | |
|---|---|---|---|---|---|---|---|
| Time | 0 | 0.5 | 1 | 2 | 3 | 4 | 5 |
| Count (CFU/ml) | 1.08x10³ | 2.44 x 10³ | 4.6x10⁴ | 6.00x10⁴ | 0 | 0 | 0 |
| Log (CFU/ml)* | 3.03±0.97 | 3.39±0.61 | 4.66±0.34 | 4.78±0.22 | 0 | 0 | 0 |

*Values are the mean±SD (n=3), SD: standard deviation, CFU: colony forming unit, AZ3: 2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole.
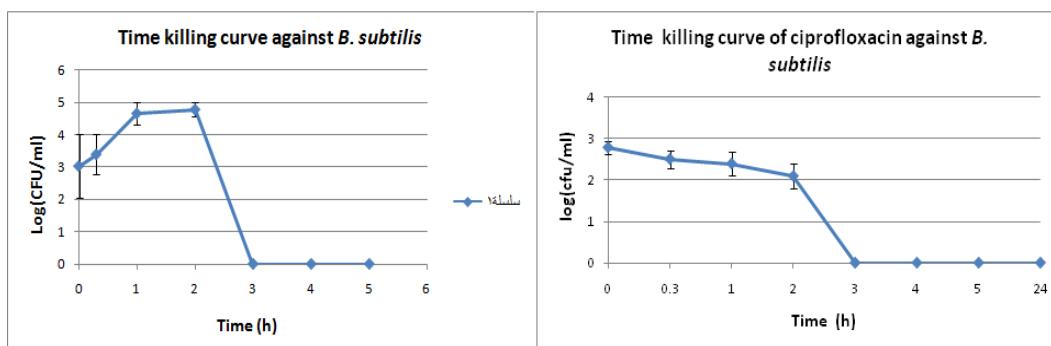


**Fig. 8: Time exposure viability curve for addition of compound AZ3 and ciprofloxacin, at MBC (125 µg/ml) and (250 µg/ml) respectively to culture of *B. subtilis* ATCC 6633**

Values are the mean±SD (n=3), AZ3: 2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole, CFU: colony forming unit, time (X-axis): time intervals needed for killing *B. subtilis*, Log (CFU/ml) (Y): viability *B. subtilis* at different times.
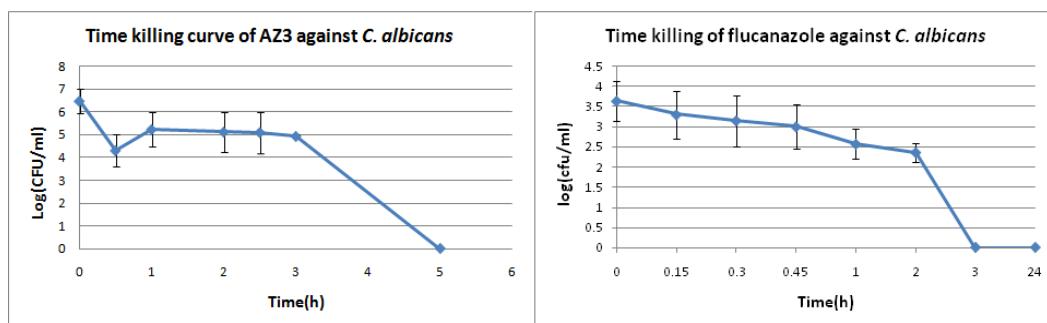
The time exposure viability curve for the addition of 0.2 ml culture of *C. albicans* ATCC 10231, with a viable count 3.0x10⁶ CFU/ml to AZ3 at 250 µg/ml dilution was demonstrated in fig. 9. The 6 log cycle reductions were obtained in approximately 5 h. The count of fungi decreased over

30 min, then the count of fungi increased slowly over the next 30 min. After 2.5 h, the count of fungi decreased slowly. The viability of microorganism decreased rapidly within the next 2 h. Accordingly, *C. albicans* was totally killed within 5 h. The LT 90% value for this experiment was approximately in the period between 3-5 h. In the time-killing test for fluconazole against *C. albicans,* the 6 log cycle reductions were detected, the curve shows that the count of bacteria decreased slowly within the first 45 min, then it decreased rapidly between 45-60 min. The viability of *C. albicans* was abolished within 3 h.

**Table 7: Bacterial time-killing of AZ3 against *C. albicans* ATCC 10231**

| microorganism | *C. albicans* | | | | | | |
|---|---|---|---|---|---|---|---|
| Time (h) | 0 | 0.5 | 1 | 2 | 2.5 | 3 | 5 |
| Count (CFU/ml) | 3.0x10⁶ | 1.98x10⁴ | 1.7x10⁵ | 1.32x10⁵ | 1.21x10⁵ | 8.6x10⁴ | 0 |
| Log (CFU/ml)* | 6.48±0.52 | 4.30±0.7 | 5.23±0.77 | 5.12±0.88 | 5.10±0.9 | 4.93±0.07 | 0 |

*Values are the mean±SD (n=3), SD: standard deviation, CFU: colony forming unit, AZ3: 2-{[4-(2,6-dimethylpiperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole.



**Fig. 9: Time exposure viability curve for addition of compound AZ3 and fluconazole, at MFC (250 µg/ml) and (500 µg/ml) respectively to culture of *C. albicans* ATCC 10231**

Values are the mean±SD (n=3), AZ3: 2-{[4-(2,6-dimethyl-piperidin-1-yl)but-2-yn-1-yl]sulfanyl}-1,3-benzothiazole, CFU: colony forming unit, time (X-axis): time intervals needed for killing *C. albicans*, Log (CFU/ml) (Y-axis): viability of *C. albicans* at different times.

## DISCUSSION

The time-killing curve test showed that, 2-{[4-(2,6-dimethyl Piperidin-1-yl)but-2-yn-1-yl]Sulfanyl}-1,3-benzothiazole (AZ3) needed much longer time, to kill *S. aureus* in comparing with the time needed to kill *B. subtilis* at MBC value 250 µg/ml; in which AZ3 needed 20-24 h to kill *S. aureus* and 2-3 h to kill *B. subtilis*. The differences in potency and efficacy of the newly synthesized compound, against *S. aureus* and *B. subtilis* may attribute to the high resistance and the high virulence nature of *S. aureus* [14]. On the other hand, ciprofloxacin needed 5 h and 3h to kill *S. aureus* and *B. subtilis* respectively, at MBC value 250 µg/ml. The time-killing curve for *B. subtilis* showed that the count of bacteria increased over the 2 h after exposure to AZ3, then the viable count of bacteria dropped sharply and became zero, this result may attribute to the endospore germination of *B. subtilis*; in which the endospore germination might increase the viable count of *B. subtilis* during the first 2 h exposure. AZ3 needed a shorter time to kill *P. aeruginosa* than the time needed to kill *E. coli,* at MBC value 250 µg/ml; whereas AZ3 needed 5 h and 3.5 h to kill *E. coli* and *P. aeruginosa* respectively. These results may be attributed to the good permeability of the synthesized compound by the dual pathway (porins and phospholipids bilayer) through *P. aeruginosa* outer membrane and the lacking porins in *E. coli* outer membrane may cause decrease in drug accumulation inside bacteria that use only one pathway for entering bacteria [11]. AZ3 killed *C. albicans* within 5 h, whereas fluconazole killed *C. albicans* within 3 h.

## CONCLUSION

The newly synthesized 2-{[4-(2, 6-dimethylPiperidin-1-yl) but-2-yn-1-yl] Sulfanyl}-1, 3-benzothiazole (AZ3) showed a promising killing activity, against *P. aeruginosa, E. coli, and C. albicans.* This study may promote further expansion of 2-mercaptobenzothiazole derivatives bearing dimethyl piperidin and other cyclic amine moieties, which may lead to compound with potent and broader antimicrobial activities.

## AUTHORS CONTRIBUTION

Msc. Aseel Alsarahni was responsible for the laboratory work and writing the paper draft

Prof. Zuhair Muhi-Eldeen is the chief author, supervised the synthesis steps

Prof. Elham Al-Kaissi was responsible for writing and supervised the microbiology part.

Msc. Heba was responsible for part of the laboratory work.

## CONFLICT OF INTERESTS

The authors have declared no conflict of interest

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