Original Article

Design, synthesis, and antibacterial evaluation of arylidene derivatives of rhodanine-3-hexanoic acid

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ABSTRACT

Background: Due to the development of resistance to various traditional antibacterial medications, bacterial infections represents a significant problem for healthcare professionals. Thus, there are unsatisfactory medical requirements for new bacteria and bacterial infections. **Rationale:** Rhodanine derivatives substituted with carboxyalkyl acid moiety have been reported to possess potent antimicrobial activity through a novel mechanism. However, their antibacterial potential has not been completely explored. **Results:** Therefore, in the present study, series of rhodanine-3-hexanoic acid derivatives bearing hydroxyl substituted benzylidene moiety at the C-5 position of the rhodanine core were synthesized through Knoevenagel condensation of rhodanine-3-hexanoic acid with various hydroxyl substituted aromatic aldehydes. All the compounds were structurally characterized and evaluated in vitro for their antibacterial activity against two Gram-positive and two Gram-negative bacterial strains. **Conclusion:** Biological data showed that synthesized derivatives exhibited potent to moderate antibacterial activity against the tested bacterial strains. The lead identified in the present study can be further explored for the design of potent antimicrobial agents.

Keywords: 2-thiazolidine-4-one, antibacterial activity, antimicrobial activity, rhodanine

INTRODUCTION

A global problem associated with high morbidity and mortality is the alarming rate of rising antimicrobial resistance in the bacterial pathogens, claiming valuable lives worldwide. In Gram-positive and negative bacteria, multidrug-resistant trends have caused traditional antimicrobial infections to be hard to treat or even untreatable in some cases.[1] As there are several health-care settings lacking in the early identification of causative microorganisms and in their antimicrobial resistance trends, wide spectrum antibiotics are generally freely and excessively prescribed for the treatment.[2] There are dramatic increases in emerging resistance and resistant bacteria can easily be spread to other patients and to the environment when combined with inadequate infection management practices. Resistance to common antimicrobial therapies by many bacterial pathogens and development of multidrug-resistant strains at an alarming rate has posted a big threat to human race.[3] Various challenges still exist in combating bacterial infections and the diseases involved which includes the existing lack of effective pharmaceuticals drugs, lack of good preventive measures, and alternative antimicrobial treatments. Moreover, a few new antibiotics are in the clinical pipeline, which may create a panic situation in the near future. The advancement of antibiotic resistance strategies is a significant global problem for the community of life sciences and public health. Scientific community is working hard to find new treatment for combating rising drug resistance.[4,5]

The rhodanine or 2-thiazolidine-4-one derivatives have been known to scientific community for over 100 years and are still under exploration due to their extremely interesting biological activity profile. The heterocyclic scaffold 2-thioxo-thiazolidin-4-one (rhodanine core) is regarded as one of the most valuable assets for the development of new drug entities, possessing numerous activities. The structural variability of 2-thioxo-1,3-thiazolidine 4-one derivatives is endowed primarily at two positions of the scaffold. The first one is the N-3, and the second

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position is C-5. The introduction of the lipophilic or hydrophilic or aromatic group at one or both of the main positions provides a range of drug design options and physicochemical parameters modifications.[6-8]

A few reports has been published which described the antibacterial potential of rhodanine derivatives bearing a carboxyalkyl acid moiety at the N-3 position of the rhodanine core. The synthesis and antimicrobial evaluation of phenolic substituted rhodanine derivatives was carried out by Kratkay *et al*. The result of antimicrobial evaluation adjudged the compound 1 as the best compound among the series of synthesized derivatives possessing minimum inhibitory concentration (MIC) values in the range of 32–62.5 μg/mL against the various tested starins. Tejchman *et al*. and Arora *et al.* reported the antimicrobial potential N-3 substituted rhodanine derivatives bearing hydroxy substituted phenyl and naphthyl moieties at C-5 position of the rhodanine core. Moreover, literature reports highlighted the contribution of lipophilic substituent at C-5 position is crucial to obtain high antibacterial potency. Inspired from the literature reports and in continuation to our previously reported work on rhodanine derivatives as antibacterial agents, we expand our work by substituting hexanoic acid chain at N-3 position and hydroxy aryl as the arylidene moiety at the C-5 position and evaluated them as possible antibacterial agents.[9-14] The design of proposed molecules is depicted in Figure 1.

RESULTS AND DISCUSSION

Chemistry

The proposed compounds were synthesized using synthetic strategy as depicted in Scheme 1. The synthesis of rhodanine-6-hexanoic acid was achieved by condensing 6-aminohexanoic acid, carbon disulfide, and sodium chloroacetate as the reactants. The rhodanine carboxylic acid was then condensed with various substituted aromatic aldehydes to yield the title compounds in good yield after purification. The FTIR spectrum of synthesized compounds exhibited a broad absorption band at 3218–3240 cm^{-1}. The characteristic peaks of due to presence of two carbonyl groups appeared at 1710–1685 cm^{-1}. The ^1H NMR spectra displayed characteristic peak due to protons of the methylene group attached to the ring nitrogen (-N-CH_2) resonated as a triplet ranging between 4.16 and 4.09 ppm. Signal due to protons of the methylene group attached to the carboxylic group appeared in the range of δ 2.38–2.30 ppm. The OH of the aryl groups appeared at 4.05–6.10 ppm while the OH of carboxyl appeared at 10–10.10 ppm. All the other characteristic peaks were also observed and found to be in good agreement with the proposed structure. Finally, the structures of the proposed compounds were confirmed using mass spectrometry and elemental analysis. Various physiochemical constants are summarized in Table 1.

Antibacterial activity

Antibacterial activity MIC was evaluated using broth dilution method against four bacterial strains, namely, *Staphylococcus aureus* (SA) and *Streptococcus pyogenes* (SP), representatives of Gram-positive bacteria, while Gram-negative strains involved in the study were *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA). Ciprofloxacin and ampicillin were used as reference drugs for the comparison of antibacterial activity. Results of antimicrobial evaluation data indicated that the compounds (9a-e) were active against various tested microbial strains as presented in Table 2. Tested compounds displayed excellent to good activity as compare to the standard drugs ampicillin and ciprofloxacin. Some compounds exhibited superior activity against tested bacterial strains as compared to ampicillin; however, all were less potent than the standard drug ciprofloxacin. A general SAR is summarized in the Figure 2.

Among the various tested compounds, 9c carrying 5-dichloro-2-hydroxy phenyl was adjudged as the most potent candidate displaying MIC ranged between 31.25 and 125 μg/mL against the tested bacterial strains. Compound 9d also showed potent activities against SA and SP (MIC = 62.5 μg/mL against both the strains) which were higher than the standard drug ampicillin. Compound 9b carrying

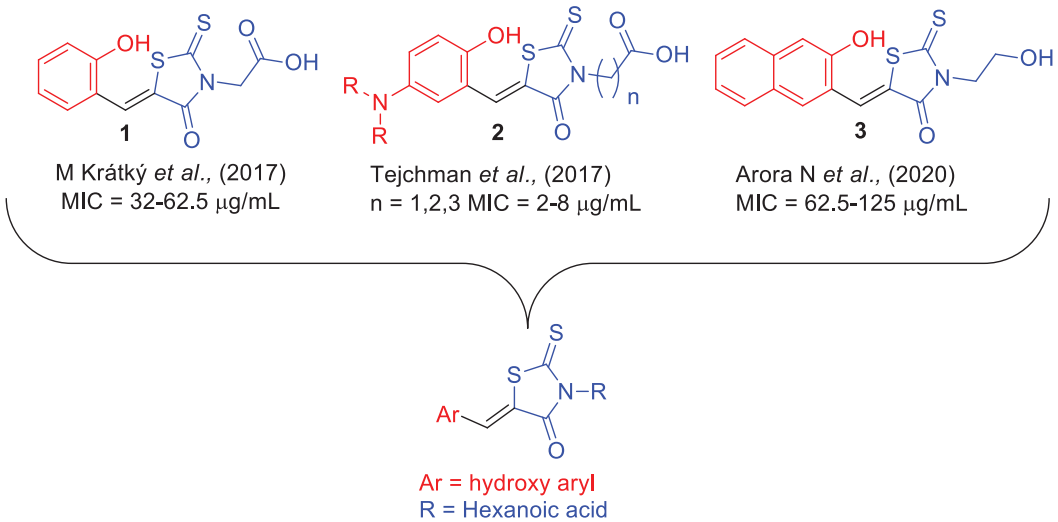
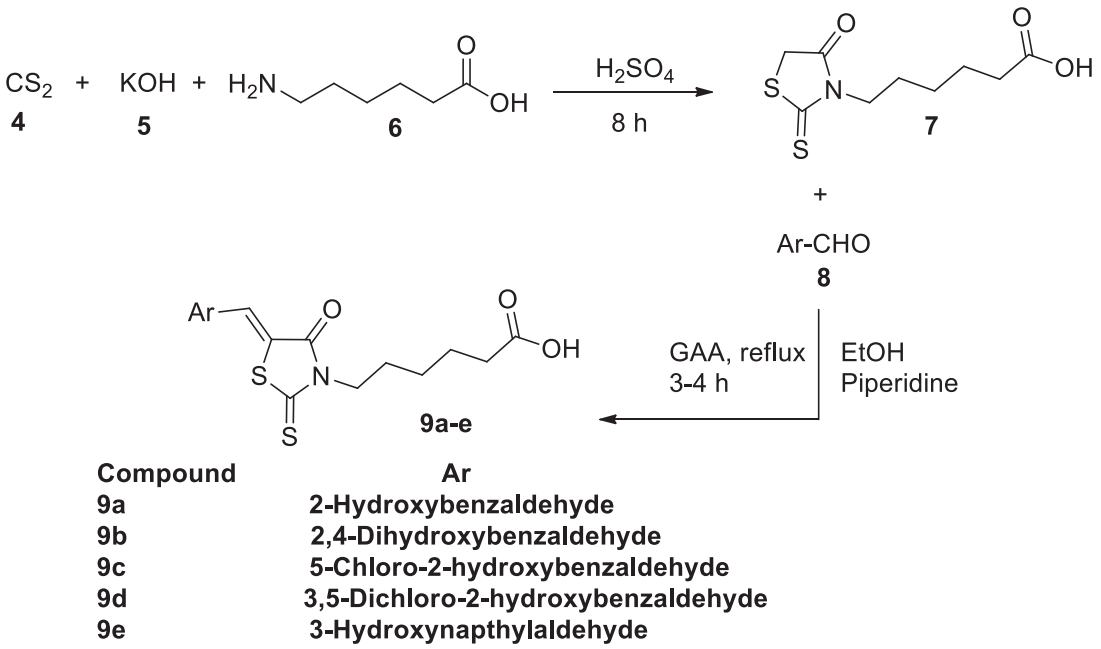


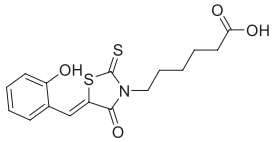
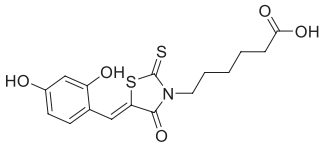
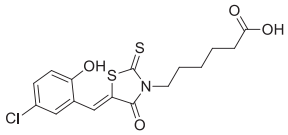
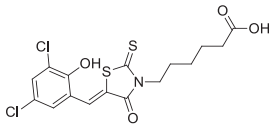
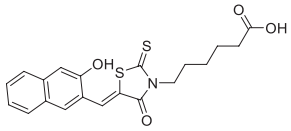
Figure 1: Design of the proposed molecules



Compound	Ar
9a	2-Hydroxybenzaldehyde
9b	2,4-Dihydroxybenzaldehyde
9c	5-Chloro-2-hydroxybenzaldehyde
9d	3,5-Dichloro-2-hydroxybenzaldehyde
9e	3-Hydroxynapthylaldehyde

Scheme 1: Synthesis of title compounds

Table 1: Physicochemical data of various title compounds (9a-e)

Compound	Structure	Color	Yield (%)	Melting Point (˚C)	Rf[a]
9a		Yellow	64	210–212	0.6
9b		Yellow	61	220–222	0.56
9c		Yellow	68	256–258	0.42
9d		Yellow	76	240–242	0.58
9e		Yellow	67	241–243	0.55

a=Hexane: Ethyl acetate (3:2)

2,4-dihydroxyphenyl was the least active derivative among the series. Compounds 9a and 9e displayed moderate inhibitory activity against the tested bacterial strains.

From the MIC studies, it was evident that nature of substituent at arylidiene phenyl moiety has impacted the activity of tested compounds. Hydroxyl phenyl (9a) and hydroxynapthyl (9e) were the weak inhibitor of tested strains. Introduction of dihydroxy phenyl in compound 9b further led to fall in the antibacterial activity and render the compound almost inactive against EC. Introduction of dichloro-substituent (9d) on the hydroxy phenyl ring improved the activity against various tested strains, while substitution with chloro-substituent (9c) at the 5-position of the 2-hydroxyphenyl ring resulted in highest antibacterial potency and provided most potent compound of the series.

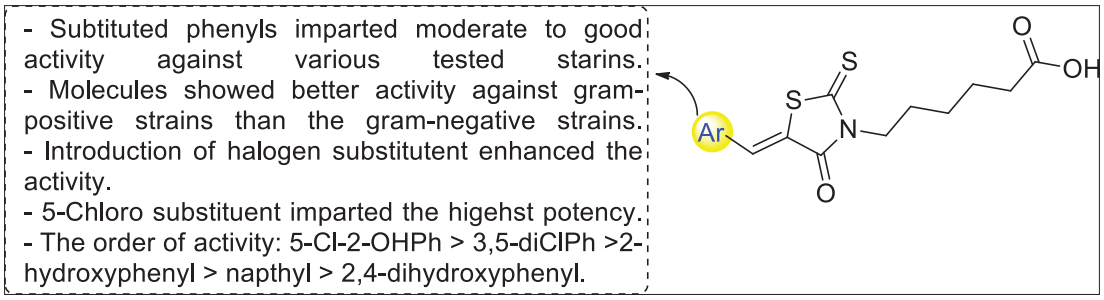


Figure 2: Structure activity relationship of newly synthesized rhodanine derivatives

Table 2: Antibacterial activity of various title compounds (9a-e)

	MIC value (μg/mL)			
Compound	**SA**	**SP**	**EC**	**PA**
9a	100	250	250	250
9b	125	250	>500	250
9c	31.25	62.5	62.5	125
9d	62.5	62.5	100	125
9e	125	250	250	250
Ampicillin	250	100	100	–
Ciprofloxacin	50	50	25	25

MIC: Minimum inhibitory concentration, SA: *Staphylococcus aureus*, SP: *Streptococcus pyogenes*, EC: *Escherichia coli*, PA: *Pseudomonas aeruginosa*

CONCLUSION

The present work is an extension to our previous work. It involves the design, synthesis, and characterization and *in vitro* evaluation studies of new rhodanine derivatives as potent antibacterial agents. Five new rhodanine hexanoic acid derivatives were synthesized, structurally elucidated, and evaluated *in vitro* for antibacterial activity. Results revealed that the synthesized compounds exhibited potent to good antibacterial activity. In particular, compounds 9c and 9d presented the most promising antibacterial profile against the tested bacterial strains with low micromolar values (MIC = 31.25–125 μg/mL) and found to be active against all the tested strains of bacteria. The antimicrobial activity was modulated by the nature of substituent attached to the 5-position of the rhodanine core. The present study identified new rhodanine hexanoic acid derivatives as promising antibacterial agents, and further studies with the identified lead molecules would be helpful in the design and development of candidates with more potent antimicrobial profile.

EXPERIMENTAL

All the intermediates and title compounds were synthesized using solution-phase chemistry. The progress of reactions was monitored by thin-layer chromatography (TLC). From the TLC, we ensured to declare the completion of the reaction. The TLC plates were visualized by viewing in UV and iodine chamber. The reaction products were purified by different work-up processes to remove unreacted starting material and impurities. Recrystallization or repeated recrystallization was done using suitable solvents to get a pure sample of title compounds. In few cases, intermediate or title compounds were purified by column chromatography. Melting points and R_f values of all the compounds and intermediates were determined. The structure and purity of the anticipated compounds were characterized by physical constants and Fourier-transform infrared spectroscopy (FT-IR) spectral studies initially followed by ^{1}H-NMR spectroscopic data, mass, and elemental analysis. The synthetic scheme consists of two steps.

General procedure for the synthesis of rhodanine-3propanoic acid [6-(4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid, 7] (Step-1).

In a 50 mL round-bottomed flask provided with a magnetic stirrer and condenser, 6-amino hexanoic acid (1 g, 11.22 mmol) was solubilized in a solution of 22% potassium hydroxide at room temperature. To this, a homogeneous solution was added carbon disulfide (0.7 mL, 11.22 mmol) drop-wise during 10 min at 25°C. After addition, peach to orange color appeared, and the contents were stirred over a period of 3 h. Now, a solution of chloroacetic acid (1.06 g, 11.22 mmol) in water was added in small portions to the reaction mixture. The yellow color appeared, and the solution was vigorously stirred at 25°C for 3 h. The resulting solution was acidified to pH 4 with concentrated hydrochloric acid, and the suspension was stirred over a period of 8 h. After this, the contents were cooled and the resulting solid was collected by filtration, washed with water, and dried under high vacuum. The crude product was purified by recrystallization from ethanol to afford crystals of pure product.

General procedure for the synthesis of arylidene derivatives of rhodanine propionic acid (9a-e, Step 2)

A mixture of rhodanine propionic acid (0.1 mmol) and substituted aromatic aldehyde (0.1 mmol) was suspended in absolute alcohol (10 mL). To this, catalytic amount of piperidine and glacial acetic acid was added. The mixture was then allowed to reflux with stirring at 80°C for 4–7 h. The completion of the reaction was monitored with the help of TLC. On completion, the precipitated solid was filtered, washed with absolute ethanol, than with aqueous ethanol solution (2 × 20 mL) and finally with water. After this, the solid was dried. Moreover, the crude product was re-crystallized with aqueous ethanol to afford pure compound in good to excellent yield.

(Z)-6-(5-(2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoicacid (9a)

IR (KBr) νmax (cm^{-1}): 3218 (O-H), 3039 (aromatic C-H stretching), 1705 and 1681 (C=O), 1619 (C=N), 669 (C=S); ^{1}H NMR (300

MHz, DMSO-d_6); δ 10 (1H, s, -COOH), 7.64 (1H, s, = CH), 7.45-7.35 (4H, m, Ar-H), 5.42 (1H,s, -OH), 4.14-4.10 (t, 2H, N-CH_2, J = 7.8 Hz), 2.38-2.33 (t, 2H, -CH_2COOH), 1.78-1.59 (m, 4H, 2 x CH_2), 1.51-1.46 (m, 2H, CH_2); mass (ESI) m/z [M+H]: 352; Elemental analysis, Calculated: C, 54.68; H, 4.88; N, 3.99; Observed; C, 54.60; H,4.82; N,4.03.

(Z)-6-(5-(2,4-dihydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid (9b)

IR (KBr) νmax (cm^{-1}): 3220 (O-H), 3045 (aromatic C-H stretching), 1702 and 1675 (C=O), 1660 (C=N), 670 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6); 10.02 (s, 1H, -COOH), 7.68 (s, 1H, =CH), 7.21-7.16 (t, 1H, J = 7.2 Hz, Ar-H), 6.84-6.77 (m, 2H, Ar-H), 10.02 (s, 1H, -OH), 4.89 (s, 1H, -OH), 4.16-4.11 (t, 2H, N-CH_2, J = 7.9 Hz), 2.38-2.33 (t, 2H, J = 7.7 Hz, -CH_2COOH), 1.76-1.61 (m, 4H, 2 x CH_2), 1.49-1.40 (m, 2H, CH_2); mass (ESI) m/z [M+H]: 370; elemental analysis, calculated: C, 52.30; H, 4.66; N, 3.81; observed: C, 52.25; H, 4.60; N, 3.78.

(Z)-6-(5-(5-chloro-2-hydroxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid (9c)

IR (KBr) νmax (cm^{-1}): 3259 (O-H), 3055 (aromatic C-H stretching), 1708 and 1683 (C=O), 1658 (C=N), 665 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6); 10.05 (bs, 1H, -COOH), 7.70 (s, 1H, =CH), 7.66 (s, 1H, Ar-H), 7.35-7.30 (m, 2H, Ar-H), 5.87 (1H,s, OH), 4.15-4.10 (t, 2H, N-CH_2, J = 7.9 Hz), 2.35-2.31 (t, 2H, J = 7.6 Hz, -CH_2COOH), 1.64-1.39 (m, 6H, 3 × CH_2). mass (ESI) m/z [M+H]: 386; elemental analysis, calculated: C, 49.80; H, 4.18; N, 3.63; observed; C,49.80, H,4.10, N, 3.60.

(Z)-3-(5-(3,5-dichloro-2-hydroxybenzylidene) -4-oxo-2thioxothiazolidin-3-yl)hexanoic acid (9d)

IR (KBr) νmax (cm^{-1}): 3228 (O-H), 3058 (aromatic C-H stretching), 1710 and 1685 (C=O), 1652 (C=N), 665 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6); 10.10 (s, 1H, -COOH), 7.65 (s, 1H, =CH), 7.66 (s, 1H, Ar-H), 7.56 (s, 1H, Ar-H), 6.01 (s, 1H, OH), 4.14-4.09 (t, 2H, N-CH_2, J = 7.8 Hz), 2.36-2.32 (t, 2H, J = 7.4 Hz, -CH_2COOH), 1.64-1.39 (m, 6H, 3 × CH_2). mass (ESI) m/z [M+H]: 419; elemental analysis, calculated: C, 45.72; H, 3.60; N, 3.33; observed: C, 45.66; H, 3.54; N, 3.53.

(Z)-6-(5-((3-hydroxynaphthalen-2-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)hexanoic acid (9e)

IR (KBr) νmax (cm^{-1}): 3242 (O-H), 3040 (aromatic C-H stretching), 1709 and 1682 (C=O), 1652 (C=N), 668 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6); 10.10 (1H, s,-COOH), 7.73(s, 1H, =CH), 7.70-7.59 (m, 6H, Ar-H), 4.05 (s, 1H, OH), 4.17-4.13 (t, 2H, N-CH_2, J = 8.1 Hz), 2.38-2.34 (t, 2H, J = 7.7 Hz, -CH_2COOH), 1.73-1.60 (m, 4H, 2 × CH_2), 1.45-1.41 (m, 2H, CH_2); mass (ESI) m/z [M+H]: 402; elemental analysis, calculated: C, 59.83; H, 4.77; N, 3.49; observed: C, 59.76, H,4.71; N, 3.42.

DETERMINATION OF MIC VALUES

The MICs of synthesized compounds were carried out by broth microdilution method as described by Rattan. DMSO was used as diluents to get the desired concentration of drugs to test on standard bacterial strains. Antibacterial activity was screened against two Gram-positive SA, SP, and two Gram-negative (EC, PA) bacteria using ciprofloxacin as a standard antibacterial agent.[15]

Mueller-Hinton broth and Sabouraud broth were used as a nutrient medium to grow for bacteria and fungus, respectively. Inoculum size for test strain was adjusted to 106 CFU per milliliter by comparing the turbidity. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of a plate of medium suitable for the growth of test organism and put for incubation at 37°C for bacteria. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting the growth of the organism was recorded as the MIC. Each test compound was diluted obtaining 1000 μg/mL concentration, as a stock solution. In primary screening 500, 250, and 125 μg/mL, concentrations of the test compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all organisms. The drugs found active in primary screening were similarly diluted to obtain 100, 50, 25, 12.5, and 6.25 μg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.[15]

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