Journal of Pharmacological Sciences 137 (2018) 283–289

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

A quinoline-based FtsZ inhibitor for the study of antimicrobial activity and synergistic effects with β-lactam antibiotics

Zhiyuan Fang [a], Li Ban [a], Yanan Li [b], Wenchang Yuan [a], Zhihua Liu [a], Ting Liu [a], Xiaomei Li [a], Kwok-yin Wong [c], Yujing Lu [d], Ning Sun [a, *], Xingang Yao [e, **]

[a] The Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou 510700, PR China
[b] Department of Pharmacy, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, 519000, PR China
[c] Department of Applied Biology and Chemical Technology and State Key Laboratory of Chirosciences, The Hong Kong Polytechnic University, Hung Hom, Kowloon, PR China
[d] Institute of Natural Medicine and Green Chemistry, School of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou 510006, PR China
[e] State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of New Drug Screening, Guangzhou Key Laboratory of Drug Research for Emerging Virus Prevention and Treatment, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, PR China

ARTICLE INFO

Article history:
Received 5 June 2018
Received in revised form
1 July 2018
Accepted 6 July 2018
Available online 17 July 2018

Keywords:
Bacterial resistance
Antibacterial activity
Synergistic effect
Cell division
FtsZ inhibitor

ABSTRACT

The antibacterial activity and the synergistic effect with β-lactam antibiotics of a new 1-methylquinolinium iodide derivative were investigated. The experimental results indicate that the compound possesses a strong antibacterial activity against a panel of bacteria including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus* and NDM-1 *Escherichia coli* with the MIC values from 0.75 μg/mL to 6 μg/mL. In addition, this compound combined with β-lactam antibiotics shows strong synergistic antimicrobial activities against antibiotic-resistant strains of *S. aureus*. The results of biochemical studies also reveal that this compound can effectively disrupt GTPase activity, polymerization of FtsZ, and cell division to cause cell death. The compound shows high potential for further development as a new generation of antibacterial agents to fight against the emergence of multidrug-resistant bacteria.

1. Introduction

Since the discovery of the first antibiotic, penicillin, by Alexander Fleming in the 1930s, the following 40 years is the “golden era” of antibiotic research and most of the antibiotics currently in use were discovered and developed in that period.[1] The discovery of antibiotics was once regarded as the ultimate victory of the battle against bacterial infections but unfortunately the development of antimicrobial resistance to common antibiotics implies that this battle is endless. Nowadays, the treatment of bacterial infections has become more difficult because bacteria can develop resistance to antibiotics at an alarming rate.[1,2] For example, Vancomycin-resistant *Enterococcus faecium* (VREF) and methicillin-resistant *Staphylococcus auerus* (MRSA) are two typical representatives of bacteria that are resistant to conventional antibiotics such as vancomycin and methicillin.[3,4] Therefore, new antimicrobial agents with novel mechanisms of action against drug-resistant bacteria are urgently needed.[2] Currently, an alternative approach that sounds effective to combat drug-resistant strains is to combine new compounds with certain existing antibacterial drugs. The key of combination therapies is to search for synergistic effects of the compounds with different mode of action, which has been widely used to treat infectious diseases. For example, β-lactamase inhibitors such as clavulanic acid and tazobactam have been successfully combined with β-lactam antibiotics to treat drug-resistant bacterial infections.[5]

Cell division is an essential process for bacterial survival. Among the bacterial cell division proteins, the filamenting temperature-sensitive mutant Z (FtsZ) is the first protein that assembles and initiates the cell division process. FtsZ is highly conserved in a wide

* Corresponding author.
** Corresponding author.
E-mail addresses: ning.sun@connect.polyu.hk (N. Sun), yaoxingang@smu.edu.cn (X. Yao).
Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2018.07.005

range of bacteria.[6] During bacterial cell division, FtsZ assembles into a highly dynamic cytoskeleton scaffold called the Z-ring by undergoing GTP-dependent polymerization, forming head-to-tail protofilaments and assembling into bundles at the site of septum formation.[7–10] The high conservation and functional importance of FtsZ in bacterial cell division render it as an attractive target for the development of novel antibacterial agents. Recently, some FtsZ inhibitors have been reported to impair the bacterial cell division.[10–20] Among them, the small-molecule PC190723 and quinuclidine **1** that exhibit potent antibacterial activity against MRSA were reported to restore the susceptibility of MRSA to β-lactam antibiotics.[21,22] Previous studies revealed that quinoline derivatives (Fig. 1), such as Zantrin Z3 and N-methylbenzofuro quinoline derivative, can effectively inhibit the proliferation of bacterial strains such as *Staphylococcus aureus* and *Bacillus subtilis* by disrupting FtsZ activity.[23–26] Herein, we reported the antibacterial activity of a new 1-methylquinolinium iodide derivative (compound **1**, 1-methyl-4-((E)-(3-methylbenzo[d]thiazol-2(3H)-ylidene)methyl) quinolin-1-ium iodide) against a panel of bacterial strains and investigated its synergistic effects in the combination with β-lactam antibiotics against antibiotic-resistant strains of *S. aureus*. In addition, we also probed the underlying mechanisms of antibacterial activity of this compound.

2. Material and methods

2.1. Antimicrobial susceptibility assay

Strains were purchased from American Type Culture Collection (ATCC, USA). Antimicrobial susceptibility tests were conducted in 96-well microplates using the broth micro-dilution procedures described in the Clinical and Laboratory Standards Institute (CLSI) guidelines.[27] The MIC was defined as the lowest compound concentration at which the growth of bacteria was inhibited by ≥90%. Three independent assays were performed for each test.

2.2. Time-killing curve assay

The growing culture of *S. aureus* ATCC BAA-41 or *Escherichia coli* ATCC 25922 was diluted to approximately 10^5 CFU mL^{-1} in volumes of Cation-adjusted Mueller Hinton broth or Mueller Hinton broth respectively, containing various concentrations of compound **1**. Cultures were incubated at 37 °C, shaking at 250 rpm. At the appropriate time intervals, 100 μL samples were removed for serial dilution in 900 μL volumes of Cation-adjusted Mueller Hinton broth and 100 μL volumes from three dilutions were spread on to MH agar. Cell counts (CFU ml^{-1}) were enumerated after incubating the plates at 37 °C for 18 h.

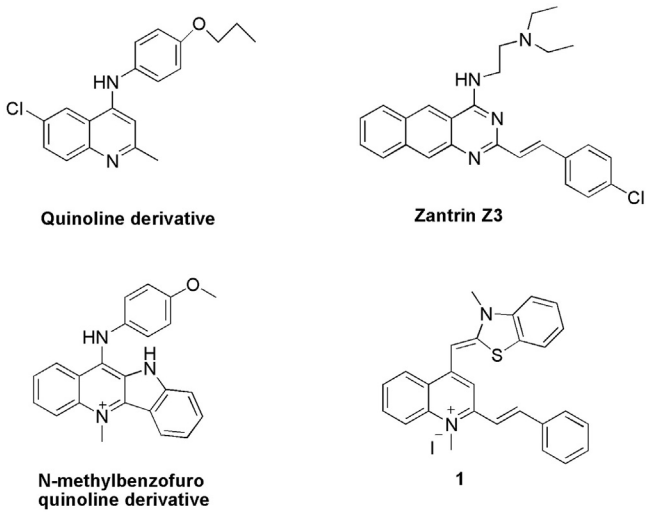

Fig. 1. Chemical structures of possible FtsZ-targeting quinoline derivatives and compound **1**.

2.3. Synergistic effect of compound *1* with β-lactam antibiotics

The synergistic activity of compound **1** and β-lactam antibiotics against ampicillin-resistant *S. aureus* ATCC 29247 and methicillin-resistant *S. aureus* (MRSA) ATCC BAA-41 were assessed using a checkerboard broth microdilution method.[28] The MICs of β-lactam antibiotics were determined as described in Section 2.1. The checkerboard test was performed in a 96-well microplate containing Mueller–Hinton broth in which a two-dimensional array of serial concentrations of compound **1** and β-lactam antibiotic is used. For example, synergy between compound **1** and methicillin was tested against MRSA in the concentration range of 1/8× MIC to 1× MIC. The synergistic effect between compound **1** and antibiotics were evaluated using the fractional inhibitory concentration index (FICI). FICI was calculated as FICI = ($MIC_{a\ combination}$ /$MIC_{a\ alone}$) + ($MIC_{b\ combination}$/$MIC_{b\ alone}$). The FIC indexes at values of ≤0.5, 0.5–1, 1–4, and >4 were defined as synergistic, partial synergistic, additive or indifferent, and antagonistic, respectively.[28] Experiments from the checkerboard test were performed in triplicates.

2.4. Visualization of bacterial morphology

The *B. subtilis* 168 cells were overnight incubated at 37 °C in 5 mL Luria–Bertani (LB) broth. Then the cells were diluted to 1 × 10^5 CFU mL^{-1} and incubated with or without compound **1** at 37 °C for 4 h. The bacterial morphology study was performed under a phase-contrast optical microscope as previously described.[25]

2.5. GTPase activity test

FtsZ protein in the biological tests was prepared as our previous study.[14] GTPase activity assay was performed as previously described to study the effect of compound **1** on the GTPase activity of FtsZ.[25]

2.6. Effect of compound *1* on the FtsZ polymerization

The light scattering assay and transmission electron microscopy analysis were performed as previously described to investigate the effect of compound **1** on the FtsZ polymerization.[25]

2.7. Molecular modeling study

The molecular modeling was performed by using CDocker program of Discovery Studio (DS) 2016. The X-ray crystal structure of *S. aureus* FtsZ was downloaded from RCSB Protein Data Bank (PDB entry: 4DXD).[22] The protein and the compound were prepared for docking using a preparation protocol of DS. Automated docking studies were carried out using DS-CDocker protocol. The top-scoring pose was visually inspected.

2.8. Preparation of compound *1*

The detailed synthesis and characterization of the compound are available in the Supporting information.

3. Results and discussion

3.1. In vitro antibacterial activity of compound 1

To determine the antibacterial activity of compound **1**, the compound was tested against a panel of clinical relevant bacterial strains and also some selected drug-resistant strains. Berberine and methicillin were tested as the reference compounds under same condition. The results are summarized in Table 1. The results showed that **1** can effectively inhibit the growth of drug-sensitive strains including *B. subtilis* 168, *S. aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, and *E. coli* ATCC 25922 with the MIC values ranged from 0.75 μg/mL to 3 μg/mL, which are comparable to that of methicillin and are much lower than that of berberine. Moreover, the antibacterial activity of **1** against the tested MRSA strains with the MIC value as low as 1.5 μg/mL is 100-fold better than that of methicillin and berberine. In addition, compound **1** also showed strong inhibitory effect on the growth of vancomycin-susceptible and vancomycin-resistant *Enterococcus faecalis* (MIC value 1.5 μg/mL) and *E. faecium* (VREs) with MIC value of 2 μg/mL, indicating that **1** is more potent than that of vancomycin (MIC values higher than 96 μg/mL against VREs).[14] For Gram-negative bacteria, the compound can inhibit the growth of *E. coli* and its antibiotics resistant mutant with the MIC value of 3 μg/mL. A multidrug-resistant *Pseudomonas aeruginosa* strain can also be effectively inhibited with **1** (MIC value of 6 μg/mL). However, the compound shows moderate antibacterial activity against multidrug-resistant *Klebsiella pneumoniae* indicated by its MIC value of 24 μg/mL. The results of antibacterial test reveal that compound **1** is potent and has a broad-spectrum antibacterial activity against most of bacterial strains including drug-resistant strains.

3.2. Time-killing curve determinations

To further investigate whether compound **1** is bactericidal or not, the time killing curves were determined by counting bacterial colonies at different time points.[27] The results of time killing curves for **1** against *S. aureus* ATCC BAA-41 and *E. coli* ATCC 25922 were illustrated in Fig. 2. Fig. 2A showed that 2× MIC and 4× MIC of **1** can rapidly reduce the viable counts of *S. aureus* below the lowest detectable limit 10^3 CFU mL^{-1} (99.9% of bacterial growth was inhibited) after incubation for 4 h. Similar effects was also found in the time-killing curves of *E. coli* cells incubated with **1** (Fig. 2B). The results also showed that 1× MIC concentration of **1** caused a ~10^2 CFU mL^{-1} reduction of *S. aureus* in 4 h and to below 10^3 CFU mL^{-1} in 8 h, and the counts were maintained under the detectable limit after 24 h incubation (Fig. 2A). On the other hand, 1× MIC concentration of compound **1** can also reduce the viable counts below 10^3 CFU mL^{-1} in the *E. coli* bacterial survival test for 24 h (Fig. 2B). These results suggested that the antibacterial activity of **1** with the same mode of bactericidal effect.

Table 1
Antibacterial activity of compound **1** against a panel of bacterial strains.

Organism	MIC (μg/mL)		
	1	Berberine	Methicillin
B. subtilis 168	1.5	96	<0.75
S. aureus ATCC 29213	1.5	192	<0.75
S. aureus ATCC 29247[a]	1.5	192	6
S. aureus ATCC BAA-41[b]	1.5	192	1024
S. aureus ATCC BAA-1717[b]	1.5	192	512
S. aureus ATCC BAA-1720[b]	1.5	192	1024
S. aureus ATCC BAA-1747[b]	1.5	192	256
S. epidermidis ATCC 12228	0.75	192	0.75
E. faecalis ATCC 29212	1.5	>192	4
E. faecalis ATCC 51575[c]	1.5	>192	4
E. faecium ATCC 49624	2	>192	4
E. faecium ATCC 700221[c]	2	>192	4
E. coli ATCC 25922	3	>192	3
E. coli ATCC BAA-2469[d]	3	>192	>1024
P. aeruginosa ATCC BAA-2108[d]	6	>192	>256
K. pneumoniae ATCC BAA-1144[d]	24	>192	>256

[a] An ampicillin-resistant strain.
[b] MRSA.
[c] Vancomycin-resistant strains.
[d] Multidrug-resistant strains.

3.3. Synergistic effects of 1 with β-lactam antibiotics

To detect whether compound **1** can restore the antibacterial activity of β-lactam antibiotics against ampicillin-resistant *S. aureus* and MRSA strains, a broth microdilution checkerboard experiment was performed. As shown in Table 2, β-lactam antibiotics showed weak or moderate antibacterial activity against MRSA with MIC values higher than 24 μg/mL. On the other hand, using β-lactam antibiotics with a combination of **1** can effectively improve their antibacterial activity against drug-resistant *S. aureus*. Compound **1** can enhance the antibacterial activity of ampicillin against ampicillin-resistant *S. aureus* from an MIC value of 24 μg/mL to 6 μg/mL, with a concentration of 0.375 μg/mL (1/4 MIC of **1**). Moreover, 0.375 μg/mL of compound **1** can dramatically improve the antibacterial activity of methicillin against MRSA (ATCC BAA-41) by reducing the MIC value from 1024 μg/mL to 32 μg/mL, with an FICI of 0.281. The combination of **1** with ampicillin or oxacillin also showed synergistic effects against MRSA with FICIs 0.5 and 0.375 respectively. In the test, compound **1** at the concentration of 0.375 μg/mL can improve the antibacterial activity of ampicillin by 4-fold (reducing MIC from 48 μg/mL to 12 μg/mL) and oxacillin by 8-fold (reducing MIC from 256 μg/mL to 32 μg/mL) against MRSA. In addition, the enhancement in antibacterial activity of imipenem and ceftazidime were observed when combining with compound **1**, with FICIs 0.75.

3.4. Effects of compound 1 on the cell morphology of B. subtilis

Some quinoline derivatives were known having antibacterial activity *via* the inhibition of FtsZ activity to cause cell elongation.[23–25] To further investigate the possible antibacterial mechanism of compound **1**, we firstly studied the cell inhibitory effect of the compound on *B. subtilis* cells. The morphology of *B. subtilis* that incubated under the conditions with and without **1** was observed through an optical microscopy. The results showed that *B. subtilis* cells with **1** were significantly elongated compared to the control (Fig. 3). The normal cell length of *B. subtilis* cells is around 5–10 μm (Fig. 3A). The cell after incubated with **1** at the MIC concentration (1.5 μg/mL), its length was found longer than 20 μm (Fig. 3B). The similar cell elongation phenomena was also found in other FtsZ inhibitors such as quinoline and berberine derivatives,[14,25,26] encouraging us to further investigate the underlying antibacterial mechanism of this compound.

3.5. Effects of compound 1 on the GTPase activity of FtsZ

Recent studies reported that quinoline derivative induced cell elongation in the antibacterial experiment may be due to their inhibitory effect on the GTPase activity of FtsZ.[23,25,26] We therefore prepared a *S. aureus* FtsZ protein and investigated the effect of compound **1** on FtsZ GTPase activity by following our previous conditions.[25] The results revealed that compound **1** can effectively inhibit the GTPase activity of FtsZ in a dose-dependent manner. In the study, compound **1** at 0.75 μg/mL showed an inhibition of about

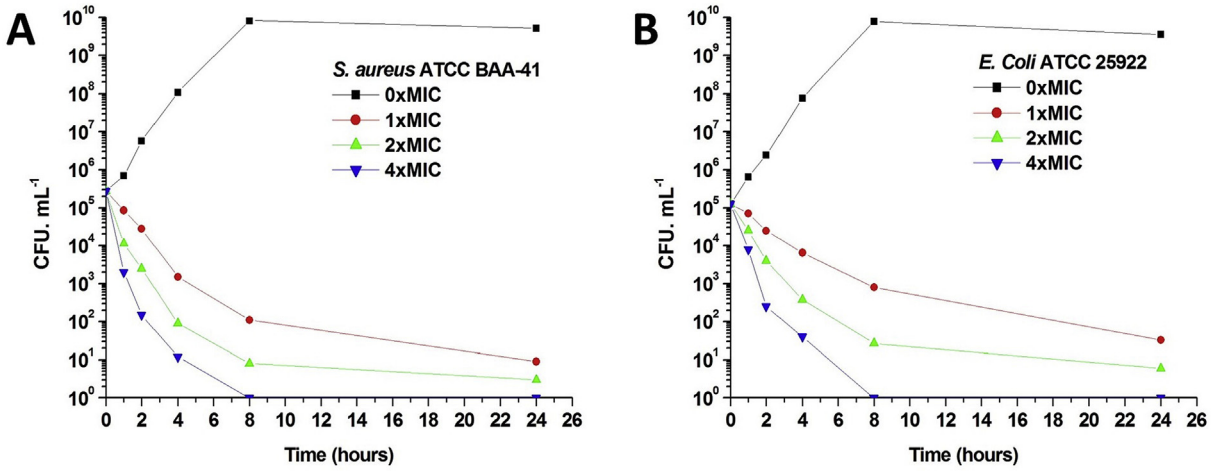

Fig. 2. Time-killing curve of compound **1** against *S. aureus* ATCC BAA-41 (A) and *E. coli* ATCC 25922 (B). Different concentrations of compound **1** were represented by different colors, 0× MIC (1% DMSO) (black), 1× MIC (red), 2× MIC (green) and 4 × MIC (blue).

Table 2
MIC values of compound 1 combined with β-lactam antibiotics against drug-resistant *S. aureus*.

Strain[a]	Compound	MIC (Single compound, μg/mL)	MIC (Checkerboard assay, μg/mL)	FICI
ATCC 29247	**1**	1.5	0.375	0.5
	Ampicillin	24	6	
ATCC BAA-41	**1**	1.5	0.375	0.5
	Ampicillin	48	12	
ATCC BAA-41	**1**	1.5	0.375	0.281
	Methicillin	1024	32	
ATCC BAA-41	**1**	1.5	0.375	0.375
	Oxacillin	256	32	
ATCC BAA-41	**1**	1.5	0.75	0.75
	Imipenem	24	6	
ATCC BAA-41	**1**	1.5	0.75	0.75
	Ceftazidime	96	24	

[a] ATCC 29247 is an ampicillin-resistant *S. aureus*; ATCC BAA-41 is an MRSA.

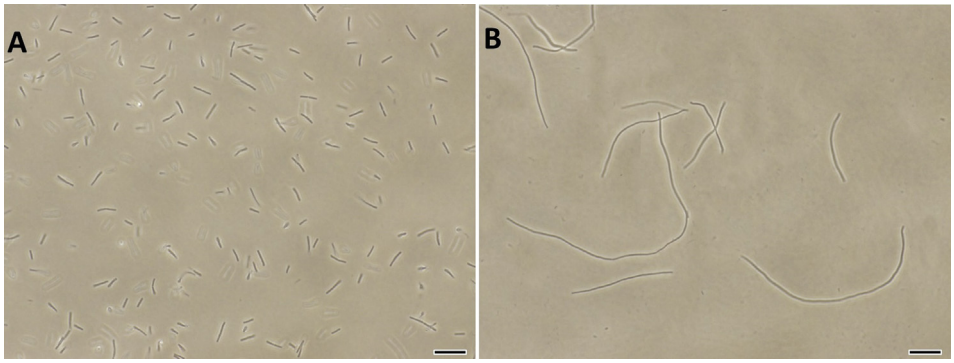

Fig. 3. The effect of compound **1** on *B. subtilis* 168 morphology. Cells of *B. subtilis* 168 were grown in the absence (A), and presence of 1.5 μg/mL of compound **1** (B). The scale bar is 10 μm.

20%. However, 50%, 70% and 80% inhibitions can be achieved by using **1** at the concentration of 1.5 μg/mL, 3 μg/mL and 6 μg/mL respectively (Fig. 4A). The results suggested that the compound inhibits bacteria growth through the inhibition of the GTPase activity of FtsZ.

3.6. *Effects of compound **1** on the polymerization of FtsZ*

Since GTPase activity of FtsZ is correlated to the dynamic polymerization of FtsZ,[29] we further investigated the impact of compound **1** on the FtsZ protein. Firstly, a light scattering assay was used to monitor the effect of compound **1** on the dynamic polymerization of FtsZ. Fig. 4B shows the time-dependent polymerization profiles of *S. aureus* FtsZ in the absence and in the presence of **1** at different concentrations ranged from 0.75 to 3 μg/mL. The results revealed that the compound enhanced FtsZ polymerization in a dose-dependent manner. The non-FtsZ-targeting antibiotic methicillin (5 μg/mL) was also tested in the same condition as a negative control and it did not show any effect on the FtsZ polymerization. In addition, the effect of compound **1** on FtsZ

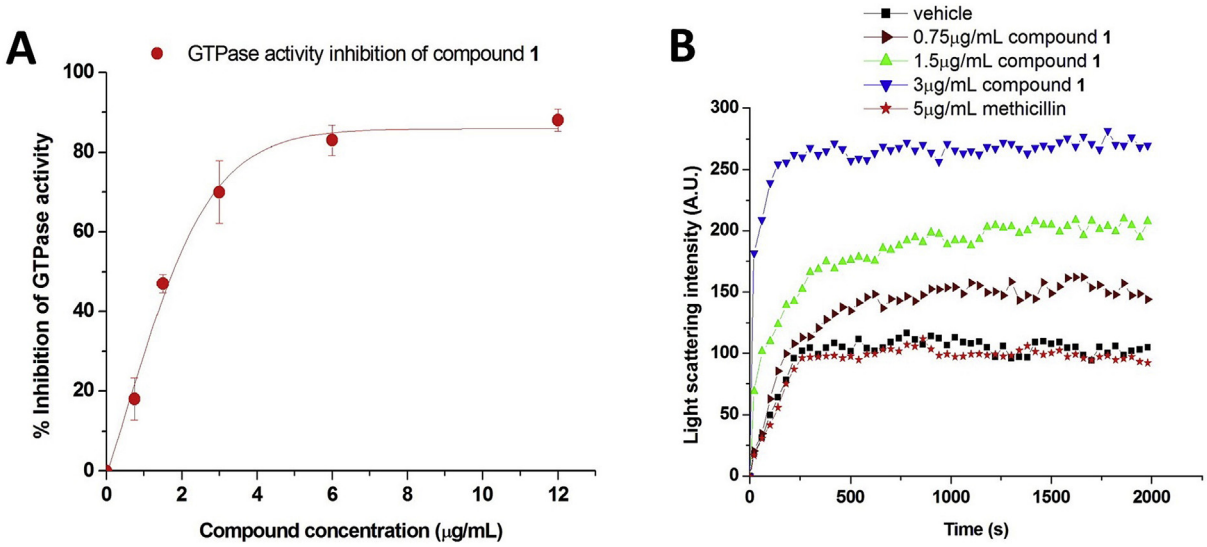

Fig. 4. Impact of compound **1** on the GTPase activity and polymerization of *S. aureus* FtsZ. (A) Inhibition of GTPase activity of FtsZ by compound **1**; (B) Time-dependent polymerization profiles of FtsZ in the absence and presence of different concentrations of compound **1**.

polymerization was observed by using transmission electron microscopy. It was found that the size of FtsZ polymers was significantly increased after incubated with compound **1** at a concentration of 1.5 μg/mL (Fig. 5). These results suggested that the antibacterial activity of compound **1** may be due to its disrupting effects on GTPase activity and polymerization of FtsZ.

3.7. Prediction of the binding mode of compound *1* in FtsZ

From the above biological assays, compound **1** is ascertained as a potent antibacterial small molecule, which is targeting FtsZ. It is of importance to further identify the possible binding site for the compound in the FtsZ protein. In the molecular modeling study, the optimal docking pose suggests that **1** probably binds into an interdomain cleft in C-terminal, which is a narrow cleft constituted by the H7-helix, T7-loop and a four-stranded β-sheet (Fig. 6A). The 2D ligand interaction diagram (Fig. 6B) shows the predicted interactions between compound **1** and the FtsZ residues. The binding of compound **1** with Asp 199, Leu 200, Met 226, Ile 228, Val 297, Leu 302 and Val 307 of FtsZ protein are through hydrophobic interactions. In addition, a hydrogen bond interaction can be found between Asp199 and hydrogen of methyl group in compound **1**. Moreover, van der Waals force is found for compound **1** interacting with a few amino acids such as Gln 192 and Gly 196 around the binding pocket.

4. Discussion

In this study, we have found that compound **1** possesses potent and broad spectrum antibacterial activity. It was also found that **1** shows remarkable synergistic effects with β-lactam antibiotics against MRSA. Moreover, this compound induced filamentation of *B. subtilis* 168 cells, which suggests that the compound inhibits bacterial cell division. In the biochemical tests, compound **1** was found to interact with FtsZ to increase the polymerization and inhibit the GTPase activity of FtsZ. The similar biological phenomena can also be found in some FtsZ-targeting compounds such as PC190723 and Zantrin Z3.[26,30] The polymerization dynamics of FtsZ is supposed to be regulated by GTPase activity of FtsZ[29] and the increment of FtsZ polymers may be due to the conformational

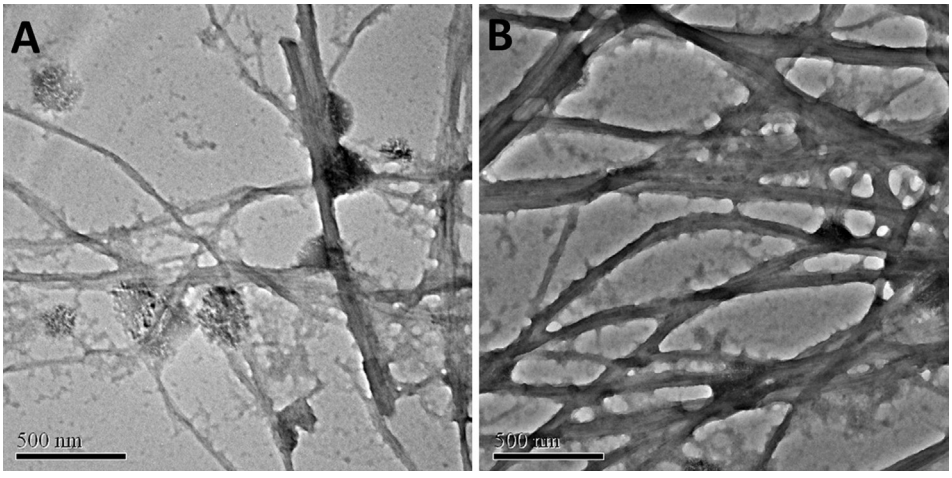

Fig. 5. Transmission electron micrographs of *S. aureus* FtsZ polymers in the absence (A) and in the presence (B) of 1.5 μg/mL of compound **1**. Scale Bar = 500 nm.

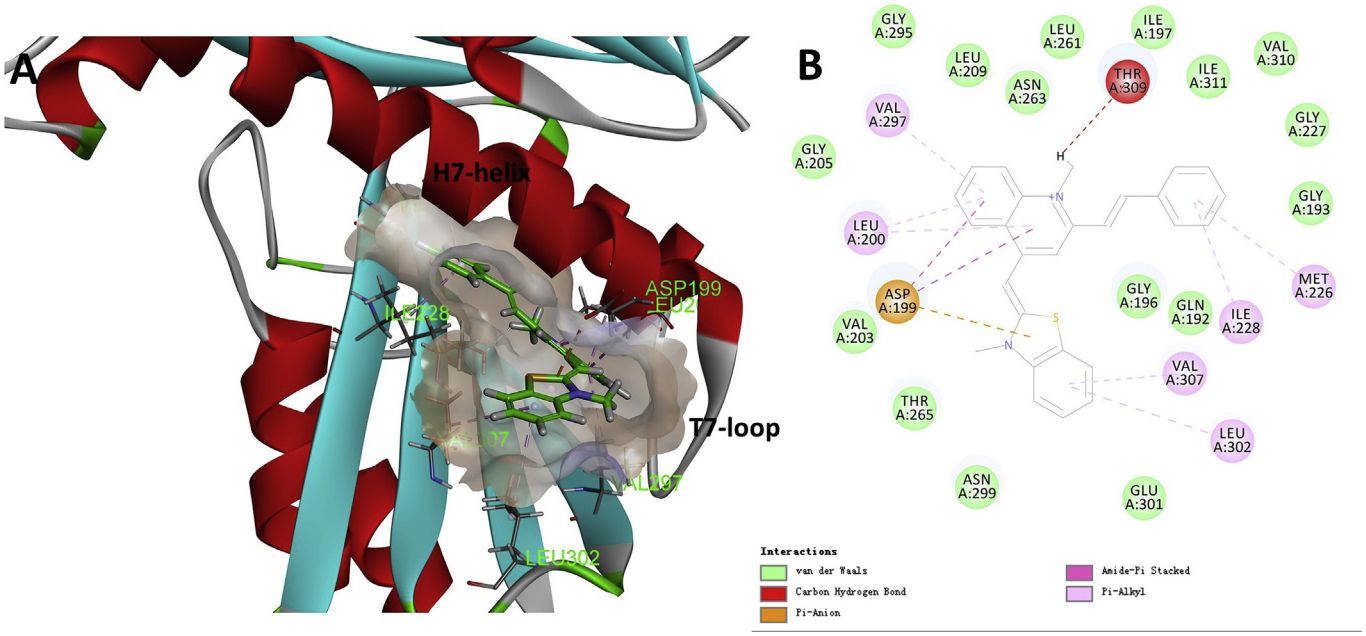

Fig. 6. Predicted binding modes of compound **1** bound to FtsZ. (A) Compound **1** bound to the interdomain cleft of FtsZ (PDB: 4DXD); (B) Predicted interaction between compound **1** and amino acids of FtsZ.

change to the high-affinity state, which enables FtsZ assembly.[31] In the antibacterial tests, compound **1** can effectively inhibit the bacterial proliferation against all Gram-positive strains in the tests including drug-resistant bacteria, such as MRSA and VREF. Low MIC values are obtained in the range of 0.75 μg/mL to 2 μg/mL, which are similar to or slightly better than some of reported FtsZ inhibitors. For example, PC 190723 can inhibit the cell division of MRSA with an MIC of 1 μg/mL.[30] Berberine derivative **2** inhibited the proliferation of MRSA and VREF with MIC values of 2 μg/mL and 8 μg/mL respectively.[14] The MIC of quinuclidine **1** against MRSA is 24 μg/mL, which is much higher than that of compound **1**.[21] On the other hand, compound **1** was found effective against some Gram-negative strains, such as *E. coli* and *P. aeruginosa*, with MIC values of 3 μg/mL and 6 μg/mL, respectively. Up to now, there are only a few FtsZ inhibitors were reported showing activity against Gram-negative strains. For instance, *N*-methylbenzofuro quinoline derivatives against *E. coli* with MIC values from 6 μg/mL to 16 μg/mL.[25] Guanidinomethyl biaryl derivative 13 against the Gram-negative strains with MIC values from 2 to 32 mg/mL.[32] The antibacterial activity on the Gram-negative strains of these compounds may be due to their ability to pass the out membrane of these bacteria. Moreover, compound **1** at the concentration of 0.375 μg/mL exhibits synergistic effects with some β-lactam antibiotics (such as ampicillin and methicillin) against MRSA. Two FtsZ inhibitors (PC190723 and quinuclidine **1**) were also reported to restore the susceptibility of MRSA to β-lactam antibiotics.[21,22] However, the mechanism still needs to be explored further. Recent study revealed that FtsZ dynamics regulates not only the rate of cell division but also the peptidoglycan synthesis.[33] Since the drug target of β-lactam antibiotics is cell wall peptidoglycan. It could be conjectured that FtsZ inhibitors disturb the polymerization dynamics of FtsZ, leading to abnormal of peptidoglycan, and making bacterial cell wall more sensitive to β-lactam antibiotics.

5. Conclusions

In conclusion, 1-methylquinolinium iodide derivative (compound **1**) was demonstrated to possess potent antibacterial activities against both Gram-positive and -negative strains including the drug-resistant bacteria such as MRSA and VREF. The compound was found to restore the susceptibility of MRSA to β-lactam antibiotics. The mode of action of the compound was also studied through biochemical evaluations. The results reveal that the compound probably binds into the interdomain cleft of FtsZ, disrupts the GTPase activity and plymerization of FtsZ, and eventually impairs bacterial cell division causing cell death. Therefore, it is worthy to develop a diversity of derivatives based on this compound and then investigate their biological activities further, particularly to study their antibacterial mechanism in-depth.

Acknowledgments

We acknowledge the support from the National Natural Science Foundation of China (81473082, 81602608, 81603118 and 81703333), Natural Science Foundation of Guangdong Province, China (2017A030313078), and Science and Technology Program of Guangdong Province (2016A020209009). NS and ZYF are also grateful to the support from Nanshan Scholar Program of Guangzhou Medical University (B185006006008 and B185006006009). This study was also supported by the Open Project of Guangdong Provincial Key Laboratory of New Drug Screening (GDKLNDS-2018OF005).

Conflict of interest

No potential conflict of interest was reported by the authors.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jphs.2018.07.005.

References

1. Arias CA, Murray BE. Antibiotic-resistant bugs in the 21st century — a clinical super-challenge. *N Engl J Med*. 2009;360:439–443.

2. Wright GD. Antibiotics: a new hope. *Chem Biol*. 2012;19:3–10.
3. Gould IM, David MZ, Esposito S, et al. New insights into meticillin-resistant *Staphylococcus aureus* (MRSA) pathogenesis, treatment and resistance. *Int J Antimicrob Agents*. 2012;39:96–104.
4. Humphries RM, Pollett S, Sakoulas G. A current perspective on daptomycin for the clinical microbiologist. *Clin Microbiol Rev*. 2013;26:759–780.
5. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. *Clin Microbiol Rev*. 2010;23:160–201.
6. Margolin W. Themes and variations in prokaryotic cell division. *FEMS Microbiol Rev*. 2000;24:531–548.
7. Li Y, Hsin J, Zhao LY, et al. FtsZ protofilaments use a hinge-opening mechanism for constrictive force generation. *Science*. 2013;341:392–395.
8. Huang Q, Tonge PJ, Slayden RA, Kirikae T, Ojima I. FtsZ: a novel target for tuberculosis drug discovery. *Curr Top Med Chem*. 2007;7:527–543.
9. Ojima I, Kumar K, Awasthi D, Vineberg JG. Drug discovery targeting cell division proteins, microtubules and FtsZ. *Bioorg Med Chem*. 2014;22:5060–5077.
10. Haranahalli K, Tong S, Ojima I. Recent advances in the discovery and development of antibacterial agents targeting the cell-division protein FtsZ. *Bioorg Med Chem*. 2016;24:6354–6369.
11. Sun N, Zheng YY, Du RL, et al. New application of tiplaxtinin as an effective FtsZ-targeting chemotype for an antimicrobial study. *Medchemcomm*. 2017;8:1909–1913.
12. Hurley KA, Santos TM, Nepomuceno GM, Huynh V, Shaw JT, Weibel DB. Targeting the bacterial division protein FtsZ. *J Med Chem*. 2016;59:6975–6998.
13. Li X, Ma S. Advances in the discovery of novel antimicrobials targeting the assembly of bacterial cell division protein FtsZ. *Eur J Med Chem*. 2015;95:1–15.
14. Sun N, Chan F-Y, Lu Y-J, et al. Rational design of berberine-based FtsZ inhibitors with broad-spectrum antibacterial activity. *PLoS One*. 2014;9:e97514.
15. Chan KF, Sun N, Yan SC, et al. Efficient synthesis of amine-linked 2,4,6-trisubstituted pyrimidines as a new class of bacterial FtsZ inhibitors. *ACS Omega*. 2017;2:7281–7292.
16. Andreu JM, Schaffner-Barbero C, Huecas S, et al. The antibacterial cell division inhibitor PC190723 is an FtsZ polymer-stabilizing agent that induces filament assembly and condensation. *J Biol Chem*. 2010;285:14239–14246.
17. Kaul M, Mark L, Zhang YZ, et al. TXA709, an FtsZ-targeting benzamide prodrug with improved pharmacokinetics and enhanced in vivo efficacy against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2015;59:4845–4855.
18. Kumar K, Awasthi D, Berger WT, Tonge PJ, Slayden RA, Ojima I. Discovery of anti-TB agents that target the cell-division protein FtsZ. *Future Med Chem*. 2010;2:1305–1323.
19. Singh P, Jindal B, Surolia A, Panda D. A rhodanine derivative CCR-11 inhibits bacterial proliferation by inhibiting the assembly and GTPase activity of FtsZ. *Biochemistry*. 2012;51:5434–5442.
20. White EL, Suling WJ, Ross LJ, Seitz LE, Reynolds RC. 2-Alkoxycarbonylaminopyridines: inhibitors of *Mycobacterium tuberculosis* FtsZ. *J Antimicrob Chemother*. 2002;50:111–114.
21. Chan FY, Sun N, Leung YC, Wong KY. Antimicrobial activity of a quinuclidine-based FtsZ inhibitor and its synergistic potential with beta-lactam antibiotics. *J Antibiot*. 2015;68:253–258.
22. Tan CM, Therien AG, Lu J, et al. Restoring methicillin-resistant *Staphylococcus aureus* susceptibility to beta-lactam antibiotics. *Sci Transl Med*. 2012;4:126ra35.
23. Mathew B, Ross L, Reynolds RC. A novel quinoline derivative that inhibits mycobacterial FtsZ. *Tuberculosis*. 2013;93:398–400.
24. Nepomuceno GM, Chan KM, Huynh V, et al. Synthesis and evaluation of quinazolines as inhibitors of the bacterial cell division protein FtsZ. *ACS Med Chem Lett*. 2015;6:308–312.
25. Sun N, Du RL, Zheng YY, et al. Antibacterial activity of N-methylbenzofuro[3,2-b]quinoline and N-methylbenzoindolo[3,2-b]-quinoline derivatives and study of their mode of action. *Eur J Med Chem*. 2017;135:1–11.
26. Margalit DN, Romberg L, Mets RB, et al. Targeting cell division: small-molecule inhibitors of FtsZ GTPase perturb cytokinetic ring assembly and induce bacterial lethality. *Proc Natl Acad Sci U S A*. 2004;101:11821–11826.
27. Wikler MA, Hindler JF, Cookerill FR, Patel JB, Bush K, Powell M. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. 8th ed. Wayne, PA.: Clinical and Laboratory Standards Institute; 2009:M07–A8.
28. Odds FC. Synergy, antagonism, and what the chequerboard puts between them. *J Antimicrob Chemother*. 2003;52:1.
29. Yang X, Lyu Z, Miguel A, McQuillen R, Huang KC, Xiao J. GTPase activity-coupled treadmilling of the bacterial tubulin FtsZ organizes septal cell wall synthesis. *Science*. 2017;355:744–747.
30. Haydon DJ, Stokes NR, Ure R, et al. An inhibitor of FtsZ with potent and selective anti-staphylococcal activity. *Science*. 2008;321:1673–1675.
31. Elsen NL, Lu J, Parthasarathy G, et al. Mechanism of action of the cell-division inhibitor PC190723: modulation of FtsZ assembly cooperativity. *J Am Chem Soc*. 2012;134:12342–12345.
32. Kaul M, Parhi AK, Zhang Y, et al. A bactericidal guanidinomethyl biaryl that alters the dynamics of bacterial FtsZ polymerization. *J Med Chem*. 2012;55:10160–10176.
33. Bisson-Filho AW, Hsu YP, Squyres GR, et al. Treadmilling by FtsZ filaments drives peptidoglycan synthesis and bacterial cell division. *Science*. 2017;355:739–743.