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ORIGINAL ARTICLE

Identification of anti-tuberculosis agents that target the cell-division protein FtsZ

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Antibiotic resistance to *Mycobacterium tuberculosis* is a growing problem. Therefore, development of new anti-tuberculosis antibiotics is urgent for the control of tuberculosis (TB) infections. FtsZ, the homolog of eukaryotic tubulin, is a GTPase that assembles into cytokinetic Z rings essential for cell division in prokaryotic cells. FtsZ (filamentous temperature-sensitive protein Z) polymerizes in a GTP-dependent manner, and polymerization of FtsZ forms into dynamic protofilaments. In this study, we screened 20 000 compounds to identify inhibitors of GTPase activity of *M. tuberculosis* FtsZ. We found that 297F inhibited GTPase and polymerization of FtsZ, and reduced the amount of FtsZ polymers. Furthermore, 297F has anti-TB activity with low cytotoxicity and shows no antibacterial activities toward other Gram-positive or Gram-negative strains. *In vitro*, 297F also induced filamentation in *Mycobacterium smegmatis*. All results suggest that 297F inhibits bacterial proliferation by targeting *M. tuberculosis* FtsZ and it may be useful as a lead compound for developing anti-TB agents.
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INTRODUCTION

Tuberculosis (TB) is a common infectious disease that claims about 2 million lives worldwide. The antibiotics currently available to treat TB are limited. Recently, HIV epidemics combined with the emergence of multidrug-resistant (MDR-TB) and extensive-resistant (XDR-TB) have aggravated a TB resurgence.[1] Thus, it is necessary to develop new antibiotics with new molecular targets to effectively treat TB.

FtsZ (filamentous temperature-sensitive protein Z) is a protein key to bacterial cell-division protein. It is present in almost all prokaryotes and is the homolog of tubulin in eukaryotes.[2] However, FtsZ shares only 10% of its sequence identity at the protein level with tubulin. FtsZ has been shown to possess GTPase activity. It binds and hydrolyzes GTP.[3,4] After GTP binding and subsequent GTPase activity, FtsZ self-assembles. FtsZ polymerizes in a GTP-dependent manner to form a 'Z ring', which is required for bacterial cell division. GTP hydrolysis and polymerization of FtsZ have been shown to be at the root of the Z-ring dynamics.[5,6] The recruitment of several other cell-division proteins leads to Z-ring contraction, resulting in septum formation and eventually cell division. In the absence of FtsZ, bacterial cell division is inhibited, although DNA replication and nucleoid segregation occur normally. Such a condition leads to a filamentous phenotype and eventually cell death. Therefore, FtsZ is an especially promising target for new antimicrobial drugs because of its central role in bacterial cell division.[7]

Mycobacterium tuberculosis FtsZ, a 40-kD protein, has been studied both functionally and structurally. Leung *et al.*[8] reported the crystal structures of *M. tuberculosis* FtsZ and its GDP and GTP complexes. The polymers of *M. tuberculosis* FtsZ are more stable and polymerize slower than *Escherichia coli* FtsZ.[9] Moreover, the low degree of sequence homology between *M. tuberculosis* FtsZ and tubulin makes it possible to find compounds specific for *M. tuberculosis*. Previous studies have shown that inhibition of FtsZ polymerization by small molecules leads to the blockage of bacterial division.[10,11] However, there are very few studies on inhibitors of *M. tuberculosis* FtsZ. Structurally modified compound SRI-3072, a tubulin polymerization inhibitor, was found to reduce the growth of *M. tuberculosis*.[12] Totarol has been shown to inhibit *M. tuberculosis*, which was determined by its ability to increase the length of *Bacilli*.[13]

In the present study, the GTPase activity of FtsZ was used to screen 20 000 compounds with different structures. We found compound 297F inhibits GTPase activity and FtsZ assembly *in vitro*, and finally resulted in cell proliferation inhibition in *Mycobacterium smegmatis*. Interestingly, 297F has anti-TB activity and is bactericidal. The low cytotoxicity to mammalian cells and high MICs on other Gram-positive as well as Gram-negative strains made 297F a promising lead anti-TB compound. In addition, our results support the idea that FtsZ may be an excellent target of new anti-TB drugs.

MATERIALS AND METHODS

Materials

GTP, isopropyl-β-D-thiogalactopyranoside, malachite green, ammonium molybdate, HEPES, fluorescein diacetate were from Sigma company

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(Sigma Aldrich, St Louis, MO, USA). Compound 297F was purchased from J&K Chemical (J&K Chemical Company, Beijing, China, synthesized by Enamine). The bacterial strains used in the MIC test were clinical isolates or purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All other chemicals used were of analytical grade.

FtsZ expression and purification

M. tuberculosis H37Rv genomic DNA was provided by the Beijing Research Institute for Tuberculosis Control. The *M. tuberculosis ftsZ* gene was amplified using primers designed from Primer 5.0. The primers used were 5′-TCCATATGACCCCCCCCGCACAACTACCTGG-3′ (*Nde*I, sense) and 5′-CG GGATCCTTAGCGGCGCATGAAGGGCGGCACGTC-3′ (*Bam*HI, anti-sense).

For the expression of *M. tuberculosis* FtsZ, the PCR product was cloned into the *Nde*I and *Bam*HI sites of pET16b. The recombinant plasmid pET16b-FtsZ was constructed, with a 6× His-tag at the N-terminal. The plasmid was transformed into the *E. coli* BL21 (DE3) strain. A single clone was grown at 37 °C in LB medium containing 100 μg ml^{-1} ampicillin. The expression of FtsZ was induced by addition of isopropyl-β-D-thiogalactoside at 30 °C for 8 h.

The supernatant containing target protein was collected and loaded onto a Ni^{2+} HisTrap chelating column (GE Health, Milwaukee, WI, USA). The protein was then eluted by a stepwise imidazole gradient in elution buffer (25 mM Tris, 500 mM NaCl, 50–500 mM imidazole, pH 7.8). Eluted fractions were analyzed by 12% (wt/vol) SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Blue staining, and the purity was also evaluated. The FtsZ concentration was measured by the Bradford method using bovine serum albumin as a standard. The purified protein was frozen and stored at −80 °C.

GTPase assay

The GTPase activity of *M. tuberculosis* FtsZ was assessed in 96-well plates by determining the ability of the enzyme to liberate inorganic phosphate from GTP. Briefly, *M. tuberculosis* FtsZ (10 μM) was incubated with increasing concentrations of GTP (0–2000 μM) in reaction buffer (50 mM Tris, 5 mM $MgCl_2$) at 37 °C for 15 min. The reaction was stopped by the addition of an acidic solution (malachite green, ammonium molybdate and polyvinyl alcohol), and then the activity was detected at 650 nm. Bovine serum albumin was used as a control. A standard curve was run concurrently with each experiment, and GTPase activity is expressed as nmol inorganic phosphate liberated per min per mg of protein.

Compound library screening

A library of 20 000 compounds was used for screening. These compounds included a combination of synthetic (synthesized by Enamine, Ukraine) and natural products (plant, actinomycetes and fungi) from the Institute of Medicinal Biotechnology. FtsZ (10 μM) was incubated with the various compounds at 37 °C for 30 min. The inhibition of GTPase activity of FtsZ was then assessed as described above, though with 1 mM GTP added. The IC_{50} was determined based on the ration of the OD units over the concentration of compounds (log plots) that fits to a variable-slope dose–response equation.

Light scattering assay

The effect of compound 297F on FtsZ assembly was monitored by a 90° light scattering assay. Briefly, FtsZ (10 μM) was mixed with different concentrations of 297F in 25 mM HEPES buffer, pH 6.5, 50 mM KCl and 5 mM $MgCl_2$ at 25 °C for 15 min. After addition of 1 mM GTP, the reagents were immediately placed in a cuvette at 37 °C. The polymerization reaction was followed by monitoring 90° light scattering at 600 nm, using a fluorescence spectrophotometer (Jasco, Tokyo, Japan, FP 6500).

Effect of compound 297F on FtsZ polymers

FtsZ (100 μM) was preincubated with different concentrations (25–100 μM) of 297F or 1% DMSO in 25 mM HEPES buffer, pH 6.5, 50 mM KCl and 5 mM $MgCl_2$ for 30 min. FtsZ was subsequently polymerized with 1 mM GTP at 25 °C for 15 min. After polymerization, the mixtures were pelleted at 227 000 *g* for 30 min. The pellets were then dissolved in SDS loading buffer. The samples were analyzed by Coomassie Brilliant Blue staining of 15% SDS-PAGE. The FtsZ bands were quantified using Quantity One software (Bio-Rad, Hercules, CA, USA).

Anti-TB activity of compound 297F

Anti-TB H37Rv (ATCC 27294) activity of compound 297F was determined using the microplate Alamar Blue assay. The activities against sensitive clinical strain STB-FJ05349 and STB-FJ05060, drug-resistant clinical isolates XDR-FJ05195 and XDR-FJ05120 were analyzed using the same method. The first-line anti-TB drugs rifampin and isoniazid were used as references. The final concentrations of each compound ranged from 0.5 to 256 μg ml^{-1}. All *M. tuberculosis* strains were cultured at 37 °C in Middlebrook 7H9 broth (Difco, Franklin Lakes, NJ, USA) supplemented with 0.2% glycerol and 10% oleic acid-albumin-dextrose-citric acid until the log-phase of growth. The cells were diluted in Middlebrook 7H9 broth to 10^6 CFU ml^{-1} with various concentrations of antibiotics. The MIC was then measured in sterile 96-well microplates, with the final volume of each well at 100 μl. The visual MIC was defined as the lowest drug concentration that prevented the color change of Alamar Blue reagent from blue to pink.

Sensitivity of other bacterial strains to 297F

The MICs of compound 297F against ATCC strains and clinical isolates were determined using the agar dilution method recommend by the Clinical and Laboratory Standards Institute (CLSI). Inoculations were adjusted to yield approximately 10^4 CFU per spot using a multipoint inoculator (Bolney, Sussex, UK) and were incubated at 35 °C for 18 h. The MICs were determined as the lowest concentration of the compound that inhibited the growth of bacteria on the plate. Levofloxacin was used as the reference drug.

Cell cytotoxicity evaluation

To determine the cytotoxicity effect of 297F, cells (MRC-5, Hep G2, HT-29, MG-63 and PC-3) were seeded in 96-well culture plates with culture medium at a density of 5×10^3 cells per well in triplicate. Cells were then subjected to treat with 297F at concentrations ranging from 0.11 to 220 μM. After the cells were grown for an additional 24 h, thiazolyl blue tetrazolium bromide reagent was added. Absorbance was then measured at 490 nm. The values were averaged from triplicate wells and IC_{50} values were calculated using concentration–response curve fitting.

Mode of action: bacteriostatic vs bactericidal mode

M. smegmatis mc^2155 is a strain close to *M. tuberculosis* but exhibits fast growing and is non-infectious. This strain was grown to early log-phase in Middlebrook 7H9 broth at 37 °C. The culture was diluted to 5×10^5 CFU ml^{-1} in fresh media containing various concentrations of 297F (0.5–32 μg ml^{-1}). Bacteria were collected at different time points, serially diluted and plated. After incubation at 37 °C for 48 h, the number of colonies was counted.

Visualization of mycobacteria morphology

M. smegmatis mc^2155 was inoculated in 7H9 broth (5×10^7 CFU ml^{-1}) containing 20 μM 297F (20 × MIC) and grown for 4 h. Then fluorescein diacetate was then added to the growing *M. smegmatis* to a final concentration of 200 μg ml^{-1}. After 10 min, cells were observed using a microscope (Nikon, Tokyo, Japan, TE2000) with a ×40 objective.

RESULTS

GTPase activity assay of FtsZ

FtsZ is known to have GTPase activity, thus we measured the activity of the *M. tuberculosis* FtsZ used in this study. First, we purified the FtsZ. The recombinant protein was expressed mainly in the soluble form when induced by isopropyl-β-D-thiogalactoside at 30 °C. After purification by Ni-NTA affinity chromatography, only one single band appeared, as determined by Coomassie Blue stained SDS-PAGE. Recombinant FtsZ protein had a molecular mass of approximately 40 kDa (Figure 1a). To determine the GTP-hydrolyzing ability of FtsZ,

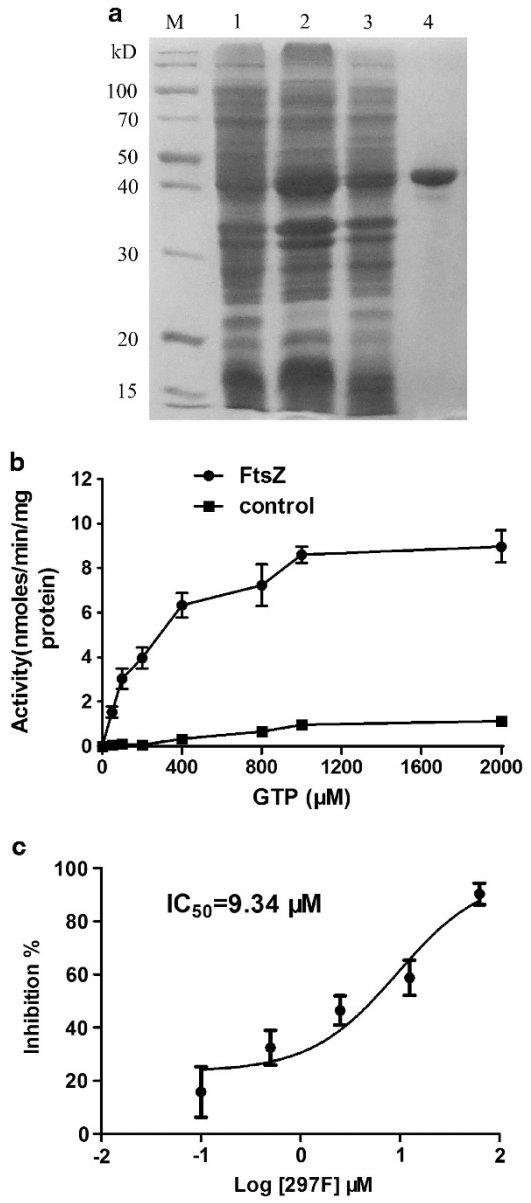

Figure 1 GTPase activity of FtsZ and inhibition of this activity by 297F. (**a**) Expression and purification of *M. tuberculosis* FtsZ. Protein samples were separated by SDS-polyacrylamide gel electrophoresis and the bands were shown after Coomassie Blue staining. Lane M, protein marker; lane 1, total cell proteins before induction; lane 2, total proteins after induction; lane 3, cellular lysate supernatant after induction; line 4, purified His-tagged FtsZ. (**b**) The GTPase activity of FtsZ was assayed (10 μM protein per reaction) at 37 °C with the indicated amounts of substrate. The specific activity of protein in nmol of inorganic phosphate liberated per min per mg is plotted against the substrate concentration. (**c**) Inhibition of GTPase activity by 297F. FtsZ (10 μM) was incubated with different concentrations of 297F for 30 min and GTPase activity was then determined as mentioned above. The IC_{50} value is plotted as the ration of the OD units over the concentration of compounds (log plots) that fits to a variable-slope dose–response equation. The experiment was repeated three times.

it was incubated with increasing concentrations of GTP (0–2000 μM). Upon addition of GTP, FtsZ showed an increase in phosphate-liberating activity compared with that of the bovine serum albumin control (Figure 1b).

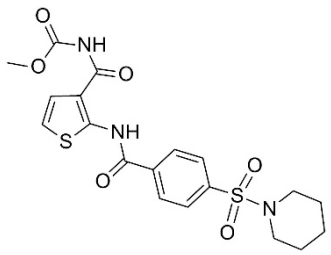

Figure 2 Structure of 297F. (methyl {2-[4-(piperidin-1-yl-sulfonyl) benzamide] thiophene-3-carbonyl}carbamate).

Compounds that inhibit the GTPase activity of FtsZ

To screen for compounds that inhibit the GTPase activity of FtsZ, various compounds at 10 μg ml^{-1} were incubated with FtsZ. From the compound library screened, we found 297F inhibited GTPase activity in a dose-dependent manner, with an IC_{50} value of 9.34 μM (Figure 1c). This indicated that the GTPase activity was strongly inhibited by 297F (methyl {2-[4-(piperidin-1-yl-sulfonyl)benzamide]thiophene-3-carbonyl}carbamate). Therefore, we chose 297F for further investigation. The structure of 297F is shown in Figure 2.

Effect of compound 297F on FtsZ polymerization

As GTP hydrolysis of FtsZ has been shown to be at the root of polymerization, we examined the effects of compound 297F on the rate and extent of FtsZ polymerization using 90° light scattering. As seen in Figure 3a, there was an increase in light scattering upon addition of 1 mM GTP, which reached a plateau at about 10 min. After 297F was added, the light scattering intensity of FtsZ was found to be remarkably decreased. We found that 50 μM 297F decreased the light scattering intensity of FtsZ assembly to almost 50% than that of the control (in the absence of compound 297F), indicating that 297F inhibits the assembly and bundling of FtsZ protofilaments. In the presence of 100 μM 297F, the initial light scattering intensity of assembly was weaker than that of the control, suggesting that 297F inhibits the initial rate of FtsZ assembly (Figure 3a).

To directly assess the effects of 297F on assembly of FtsZ, we measured the amount of steady-state polymer mass. Specifically, we examined whether 297F could prevent the assembly of FtsZ in the polymerization buffer. The results showed that 50 μM 297F significantly reduced the polymers of FtsZ in sediment (Figure 3b). The polymers observed in SDS-PAGE were reduced over 50% in the presence of 100 μM 297F. Therefore, 297F reduced the sedimentation of FtsZ in the reactions, indicating a destabilizing effect of 297F on polymer assembly.

Anti-TB activity of compound 297F

We speculated that the GTPase activity of FtsZ would be essential for cell division and its inhibition would lead to the death of *M. tuberculosis*. Indeed, compound 297F showed anti-TB activity with a MIC of 4.4 μM for the standard strain H37Rv (Table 1). In wild-type clinical strains (STB-FJ05349 and -FJ05060), the MIC range was 8.8–35.2 μM. For the clinical MDR strains (XDR-FJ05195 and -FJ05120), 297F showed a potency with a range 17.6–35.2 μM. This indicated that 297F had anti-TB activity comparable to the first-line drugs.

Because we used *M. tuberculosis* FtsZ for the drug screen, the identified compound may show stronger growth inhibition of this species. Thus, we examined the growth inhibition of 13 bacterial strains from both clinical isolates and the ATCC by 297F. Results

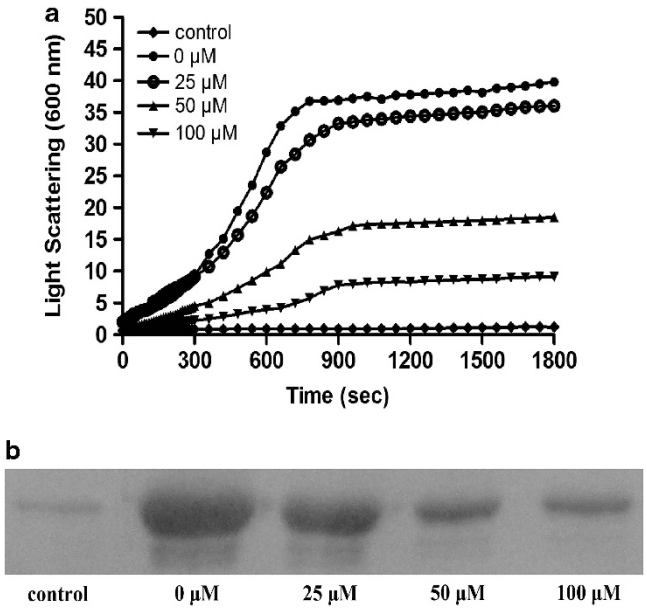

Figure 3 Effect of 297F on FtsZ polymerization. (**a**) 297F inhibited the rate and extent of FtsZ assembly. FtsZ (10 μM) was polymerized with 25 mM HEPES, 50 mM KCl, 5 mM $MgCl_2$ and 1 mM GTP in the absence or presence of different concentrations (0–100 μM) of 297F. The control indicates FtsZ polymerizes without GTP and compound. The rate and extent of the assembly reaction were monitored at 600 nm. (**b**) 297F prevented assembly of FtsZ polymers. FtsZ (100 μM) was preincubated with different concentrations (0–100 μM) of 297F. FtsZ polymerizes without compound and GTP was shown as the control. After addition of 1 mM GTP for 15 min, the polymers were pelleted at 227 000 *g* for 30 min. The pellets were then analyzed by 15% SDS-polyacrylamide gel electrophoresis and the FtsZ bands were visualized by Coomassie Brilliant Blue staining.

Table 1 MICs of 297F against various strains

	MIC (μM)			
Strain	*297F*	*Rifampin*	*Isoniazid*	*Levofloxacin*
H37Rv	4.4	0.3	1.8	—
STB-FJ05349	8.8	0.6	3.6	—
STB-FJ05060	35.2	0.6	3.6	—
XDR-FJ05195	35.2	>311	29.2	—
XDR-FJ05120	17.6	>311	29.2	—
Staphylococcus aureus 29213[a]	>70	—	—	0.67
Staphylococcus aureus 33591[b]	>70	—	—	0.67
*Staphylococcus epidermidis*12228[c]	>70	—	—	21.6
*Staphylococcus epidermidis*12-10[d]	>70	—	—	10.8
Enterococcus faecalis 29212[e]	>70	—	—	2.7
Enterococcus faecalis 51299[f]	>70	—	—	1.3
Enterococcus faecium 700221[f]	>70	—	—	172.8
Enterococcus faecium 09-10[e]	>70	—	—	172.8
Escherichia coli 25922[g]	>70	—	—	⩽0.1
Escherichia coli 09-1[h]	>70	—	—	10.8
Klebsiella pneumonia 700603[h]	>70	—	—	5.4
Klebsiella pneumonia 09-8[g]	>70	—	—	10.8
Pseudomonas aeruginosa 27853	>70	—	—	10.8

STB, clinically sensitive strain of *M. tuberculosis*; XDR, extensive drug resistance of *M. tuberculosis*. Strain FJ05349, FJ05060, FJ05195 and FJ05120 were all clinical isolates of *M. tuberculosis*.
[a]MSSA, methicillin-sensitive *Staphylococcus aureus*.
[b]MRSA, methicillin-resistant *Staphylococcus aureus*.
[c]MSSE, methicillin-sensitive *Straphylococcus epidermidis*.
[d]MRSE, methicillin-resistant *Straphylococcus epidermidis*.
[e]VSE, vancomycin-sensitive enterococcus.
[f]VRE, vancomycin-resistant enterococcus.
[g]ESBLs(−), extended spectrum β-lactamases (−).
[h]ESBLs(+), extended spectrum β-lactamases (+).

showed that 297F had high MICs on both Gram-positive and Gram-negative strains (MIC > 70 μM; Table 1).

Moreover, the IC_{50} of cytotoxicity for normal lung fibroblast MRC-5 cells was found to be 55 μM. For cancer cells Hep G2, HT-29, MG-63 and PC-3, the IC_{50} were all ⩾55 μM. These indicated that the compound 297F exhibited promising anti-TB activity without appreciable cytotoxicity.

Mechanism of *M. tuberculosis* growth inhibition by 297F

Compound 297F may inhibit the growth of TB through bacteriostatic or bactericidal effects. As *M. tuberculosis* grows slowly, we chose *M. smegmatis*, a nonpathogenic strain biologically close to *M. tuberculosis*, to examine viability after treatment with 297F. The MIC of 297F for *M. smegmatis* was 1.1 μM. Interestingly, 297F showed bactericidal activity against this strain at the MIC and the activity was increased dramatically when the concentrations were at 4×, 16× and 64× MIC (Figure 4). The results suggest a strong bactericidal activity of 297F against mycobacteria.

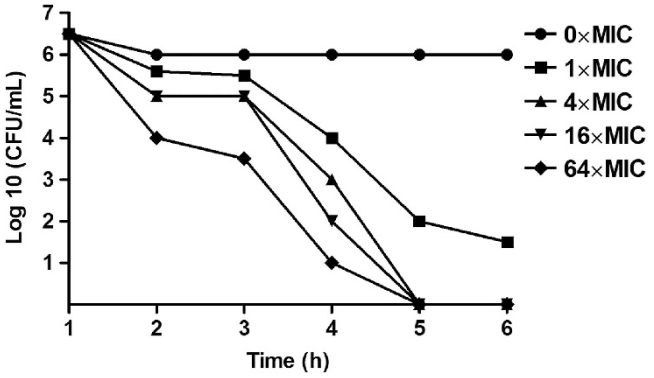

Figure 4 Dose-dependent bactericidal activity of 297F against *M. smegmatis* mc^2155. The MIC of 297F against this strain is 1.1 μM. Viability of *M. smegmatis* mc^2155 was determined in the presence of 297F at 1- to 64-fold of MIC.

297F inhibited cell proliferation

As 297F inhibits the assembly and bundling of FtsZ protofilaments, we speculate that it may induce filamentation in mycobacteria. Fluorescein diacetate is a cell dye that can enter the integral cell membrane.[14] Thus, we examined the effect of 297F on the morphology of *M. smegmatis* by comparing the fluorescence of fluorescein diacetate on the strain in the absence and presence of 297F. Treatment of 297F increased the length of *M. smegmatis*, with elongation of two- to threefold (3–4 to 7–8 μm) (Figure 5). Normally, the assembly of FtsZ forms the backbone of the Z ring. 297F may inhibit the polymerization of FtsZ into the Z ring, resulting in an imperfection in septum formation (cytokinesis). In addition to the segregated nucleoids, the *M. smegmatis* showed a remarkable elongation. Therefore, the proliferation of *M. smegmatis* was inhibited by 297F.

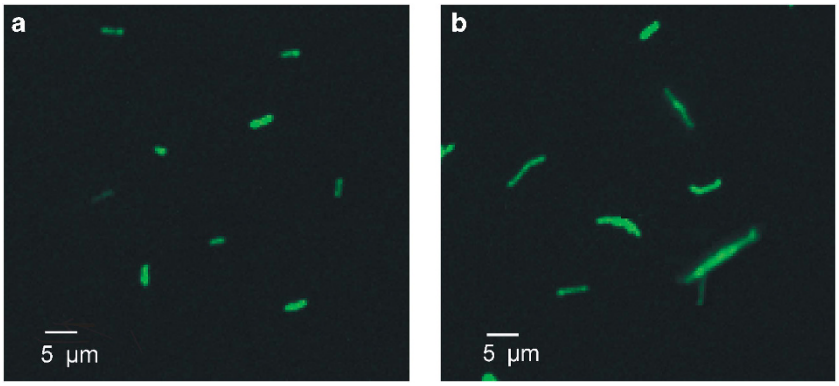

Figure 5 297F induced filamentation in *M. smegmatis* mc^{2}155. Cell morphology was observed under a fluorescence microscope. *M. smegmatis* mc^{2}155 was grown 4 h in the absence (**a**) and presence of 20 μM 297F (**b**). The scale bar is 5 μm.

DISCUSSION

Development of new anti-TB agents is of critical importance worldwide. Cell division is considered to be one of the important therapeutic targets for antibacterial drugs.[15] FtsZ is a protein crucial to bacterial cell division.[16] In the present study, we focused on FtsZ from *M. tuberculosis*. By analyzing a compound library, we first found that compound 297F inhibited GTPase activity of FtsZ. Additionally, light scattering and SDS-PAGE results from this study suggest that 297F decreased the FtsZ polymers. More importantly, 297F showed anti-TB activity and had high MICs on other bacterium. Furthermore, 297F inhibited bacterial proliferation *in vitro*. Collectively, our results suggest that we have identified an anti-TB compound that targets cytoplasmic protein FtsZ.

There are many reports on broad-spectrum inhibitors of FtsZ.[7,17,18] Because FtsZ is absent from the mitochondria of higher eukaryotes, it has evolutionary distance from tubulin, and a known biochemical activity and atomic structure, FtsZ is an attractive target for developed agents that may cause selective bactericidal effect to bacterial pathogens.[12,19] However, there are few selective inhibitors of *M. tuberculosis* known to date. It was previously found that totarol increased the length of *B. subtilis*, suggesting it has inhibitory activity against mycobacterial. Consequently, researchers confirmed that totarol indeed inhibited the proliferation of *M. tuberculosis*.[13,20] Both SRI-3072 and SRI-7614 are 2-alkoxycarbonylaminopyridines, belonging to a library of tubulin inhibitors. Compounds in this library were previously applied to a *M. tuberculosis* strain to test for anti-TB activity, and ultimately SRI-3072 and SRI-7614 were found to have activity.[12] To our knowledge, there are no anti-TB agents by screening inhibitors of GTPase activity of *M. tuberculosis* FtsZ until now. The advantage of the screening method used in our study is more likely to find selective inhibitors.

To our surprise, 297F was found to selectively inhibit the growth of *M. tuberculosis*. As is known, FtsZ is ubiquitous in prokaryotes and archaea, and these organisms all have basically the same structures. Previous studies have shown that FtsZ has two domains, one of which is a GTPase domain and the other is a water-polarizing residue, and the two domains are arranged around a central helix.[2,8,21] Sequence alignment of the FtsZ structure revealed that most residues involved in GDP binding are conserved, which is different from that found in GTPase. GTP binding of FtsZ involves four phosphate-binding loops and a sugar-binding loop in the first domain, with guanine being recognized by residues in the central connecting helix. Although the structures of FtsZ in bacteria are similar, there are some significant differences between those of *E. coli* and *M. tuberculosis*. The *M. tuberculosis* FtsZ polymerizes and depolymerizes much more slowly than *E. coli*, and the formed polymers are more stable.[22] Further, divalent calcium strongly enhances the assembly of *E. coli* FtsZ, but has no effect on *M. tuberculosis* FtsZ.[9] These findings indicate that the assembly dynamics of *M. tuberculosis* FtsZ are regulated by different mechanisms than other bacteria. We infer that the highly selective activity of 297F against *M. tuberculosis* might originate from this diversity.

Among the known anti-TB agents, totarol inhibited the polymerized mass of *M. tuberculosis* by 27% at 50 μM with a MIC of 2 μM.[13,20] SRI-3072 and SRI-7614 inhibited *M. tuberculosis* GTPase activity by 20–25% at 100 μM with a MIC of 0.15–6.25 μg ml^{-1}.[12] In this study, 297F inhibited the GTPase activity of FtsZ with an IC_{50} value of 9.34 μM and decreased the FtsZ assembly to almost 50% at 50 μM. Thus, the anti-TB activity and selectivity of 297F is comparable to that of the known agents. In addition, there was no biological activity report on 297F in the previous studies.

The GTPase activity of *M. tuberculosis* FtsZ was inhibited by 50% in the presence of 9.34 μM 297F, whereas the growth of *M. tuberculosis* H37Rv was inhibited with a MIC of 4.4 μM. It seems that 297F required to inhibit *M. tuberculosis* proliferation was lower than the concentration required to inhibit either GTPase activity or assembly of FtsZ *in vitro*. A similar difference was observed in several reports.[13,23,24] The possible reason for this might be that the binding of 297F with FtsZ interferes with the binding of FtsZ with its interacting proteins, such as ZipA, FtsA, FtsW.[25–27] All these interactions could also inhibit the formation and functioning of the cytokinetic Z ring in bacteria. Another possible explanation is that the intracellular concentration of 297F is likely to be much higher than the concentration of the compound *in vivo*.

We found 297F inhibits the assembly and bundling of FtsZ protofilaments, and increases the cell length of *M. smegmatis*, suggesting it has anti-proliferative activity. In the bacteria, cell division is engineered by the dynamic of Z ring.[28] Recent studies indicated that compounds targeting FtsZ assembly may perturb the Z ring to inhibit bacterial cytokinesis.[13,29] We speculate that 297F also perturbes the Z-ring formation. However, further investigation is needed to demonstrate this. DNA condensation and nucleoid segregation are also known to be key biological processes in cell division. Several reports have found that inhibitors of FtsZ GTP activity did not perturb DNA condensation and nucleoid segregation.[13,24,29] In future studies, we are going to determine whether 297F affects DNA condensation and nucleoid segregation, contributing to its division inhibition specificity of the Z ring.

Tubulin, the eukaryotic homolog of FtsZ, has been successfully used as a target for developing drugs against several diseases, including cancer and fungal diseases.[30,31] The fact that FtsZ is an essential protein in almost all the prokaryotes makes it an attractive target for developing novel antimicrobial agents. In this study, we found 297F inhibited GTPase activity and assembly of FtsZ and it was selective to *M. tuberculosis* and displayed low toxicity to mammalian cells. Therefore, it is possible that 297F may serve as a lead for antibiotic development through chemical modification to optimize inhibitor potency and specificity.

ACKNOWLEDGEMENTS

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