Electronic Supporting Information

Antibacterial activity evaluation and mode of action study of novel thiazole-quinolinium derivatives

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1. Visualization of bacterial morphology

Fig. S1. Morphology analysis of *B. subtilis* 168. Cells were grown in the presence of 4a1-4a3 and 4b1-4b4. Scale bar=20 μm.

2. Visualization of bacterial cell membrane

The perturbation of the membrane structure can also promote cell lysis or even trigger cell death. Compounds 4a1, 4a4, 4b1 and 4b4, which possess strong antibacterial activity and cell division inhibitory effect, were subjected additional morphological studies to investigate their effects on cell membrane structures by using *B. subtilis* cells as a model. A commercial membrane stain FM 4-64 was used for imaging. From Fig. S2 and Fig. S3, it revealed that the compounds did not cause any significant changes on the cell membrane of *B. subtilis* cells.

Fig. S2. Visualization of cell membrane of *B. subtilis* stained with red fluorescent dye FM4-64 in the absence (A) and presence of 4a4 (B). Scale bar=20 μm.
Fig. S3. Visualization of cell membrane of \textit{B. subtilis} stained with red fluorescent dye FM4-64 in the presence of 4a1, 4b1, 4b4. Scale bar=20 μm.

3. Light-scattering assay of 4b4, 4e1 and 4e3

Fig. S4. Effect of 4e1, 4e3 and 4b4 on the polymerization of FtsZ at a concentration of 0.125-0.5 μg/mL.
4. GTPase activity assay of 4a4 and 4b4

The dynamic assembly of FtsZ is strictly regulated by its GTPase activity [1, 2]. Two compounds, 4a4 and 4b4 were selected to explore their potential in disrupting GTPase activity of FtsZ. The results showed that these compounds did not have any significant effect on the GTPase activity of SaFtsZ (Fig. S5). As a matter of fact, same phenomenon also occurs in the reported FtsZ-inhibitor 2,6-difluoro-3-aminobenzamide derivative [3] and a conversion product of PC190723 [4]; these compounds were reported to bind to the interdomain cleft of FtsZ without interfering GTPase activity.

![Graph showing inhibition of GTPase activity of FtsZ by compounds 4a4 and 4b4.](image)

Fig. S5. Inhibition of GTPase activity of FtsZ by compounds 4a4 and 4b4.

5. Visualization of Z-ring in bacterial cells

![Images showing perturbation of cytokinetic Z-ring in B. subtilis.](image)

Fig. S6. The perturbation of the cytokinetic Z-ring in B. subtilis. Cells of B. subtilis were grown in the presence of 4a1, 4b1, 4b4. Scale bar=10 μm.
6. Hemolytic activity of 4a4 and 4b4

Hemolytic activity of compounds 4a4 and 4b4 was conducted using human erythrocytes. While hemolysis rate of more than 5% indicates break down of erythrocytes [5], results showed that these compounds did not reflect significant hemolysis effect. The hemolysis rates of 4a4 and 4b4 at 32×MIC (MICs for S. aureus ATCC 29213 were 1 µg/mL and 2 µg/mL, respectively) were lower than 5%, and in the previous reports [6], the cells treated by Triton X-100 (0.002 to 1%) were completely hemolyzed under the same conditions, suggesting that compounds 4a4 and 4b4 did not display cytotoxicity against human erythrocytes.

![Hemolytic activity graph](image)

**Fig. S7.** Hemolytic activity of compound 4a4 and 4b4. Human erythrocytes were treated with compounds 4a4 and 4b4 (0.125–64 µg/mL).

7. Drug resistance study of 4a4 and 4b4

![Drug resistance graph](image)

**Fig. S8.** Bacterial resistance study of compound 4a4 and 4b4 against B. subtilis 168.
Fig. S9. Bacterial resistance study of compound 4a4 and 4b4 against *E. coli ATCC* 25922.

8. Molecular modeling studies of 4a4 and 4e1 with FtsZ protein
**Fig. S10.** (A) Molecular modeling studies of 4a4 (green), 4b4 (yellow) and 4e1 (purple) with FtsZ protein; (B) Predicted interactions between 4b4 and the amino acids of FtsZ; (C) Predicted interactions between 4e1 and the amino acids of FtsZ.
9. $^1$H NMR, $^{13}$C NMR and HRMS spectra of compounds 4a1, 4a3-4a4, 4b3-4b4 and 4c1-4e4

Fig.S11. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4a1.
Fig. S12. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4a3.
Fig. S13. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4a4.
Fig. S14. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of
Fig. S15. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4b3.
compound 4b4.
Fig. S16. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4c1.
Fig.S17. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and ESI-MS spectra of compound 4c2.
Fig.S18. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4d1.
Fig.S19. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4d2.
Fig. S20. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and ESI-MS spectra of compound 4d3.
Fig.S21. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4e1.
**Fig.S22.** $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4e2.
Fig.S23. $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound 4e3.
**Fig. S24.** $^1$H NMR (DMSO-$d_6$), $^{13}$C NMR (DMSO-$d_6$) and HRMS spectra of compound **4e**.
References