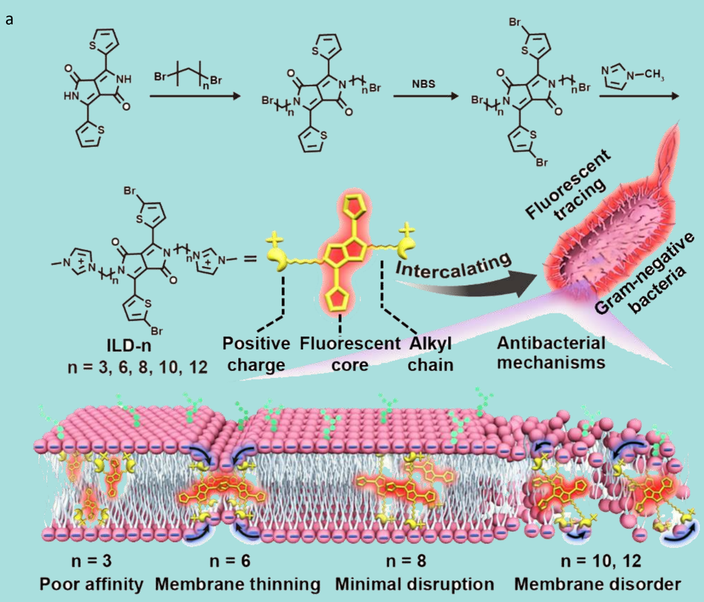
Structure-Antibacterial Activity Relationships of Ionic Liquid Derivatives against Gram-negative Bacteria

1st Liang Zheng,[a] 1nd Manman Yu,[a] 1rd Jing Li,[b] 4th Bingran Yu,[a,*] 5th Fu-Jian Xu[a,*]

[a]*Beijing Laboratory of Biomedical Materials,* [b]*Beijing Advanced Innovation Center for Soft Matter Science and Engineering, Beijing University of Chemical Technology, Beijing, 100029, China*

**Corresponding author: yubr@mail.buct.edu.cn (B. Yu), xufj@mail.buct.edu.cn (F. J. Xu)*

INTRODUCTION



Scheme1. Synthesis of fluorescent ILDs and their presumed interaction mechanism on bacterial membrane.

Ionic liquid derivatives (ILDs) have broad-spectrum antibacterial activities, but the unclear antibacterial mechanism inhibits their further development and application, especially in Gram-negative bacteria, which has highly organized structures of cell membranes. Herein, we designed a series of flexible fluorescent diketopyrrolopyrrole based on ILDs with various alkyl chain lengths. And the structure-antibacterial activity relationships of ILDs against *Escherichia coli* (*E. coli*) were systematically studied thorough agar plate count, fluorescent tracing, morphology, molecular biology and molecular dynamics simulations. More importantly, the similar structure-antibacterial activity relationships were demonstrated appropriate for another gram-negative bacteria PAO1. And both ILD-6 and ILD-12 had significant in vivo therapeutic effects on the PAO1-infected rat model.

EXPERIMENTS

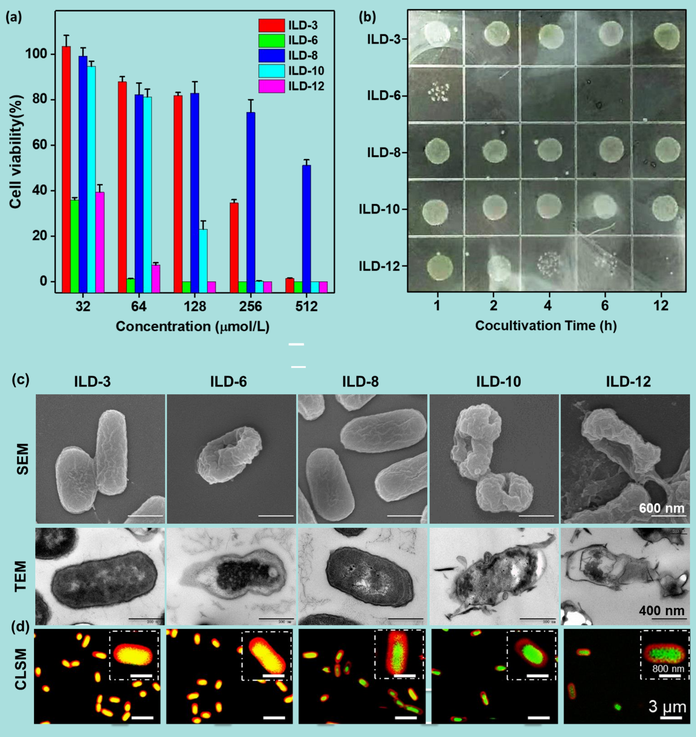


Figure 1. (a) Cell viability of *E. coli* ATCC25922 incubated with ILDs of various concentrations for 12 h. (b) Bacterial colony photos of *E. coli* ATCC25922 incubated with 128 μmol L^{-1} of ILDs for different time. (c) SEM, AFM and TEM images of *E. coli* ATCC25922 after 12 h treatment with 128 μmol L^{-1} of ILDs. (d) CLSM images of *E. coli* ATCC25922 treated with 64 μmol L^{-1} of ILDs for 4 h (red for ILDs, green for DAPI, and yellow for the merged color of red and green).

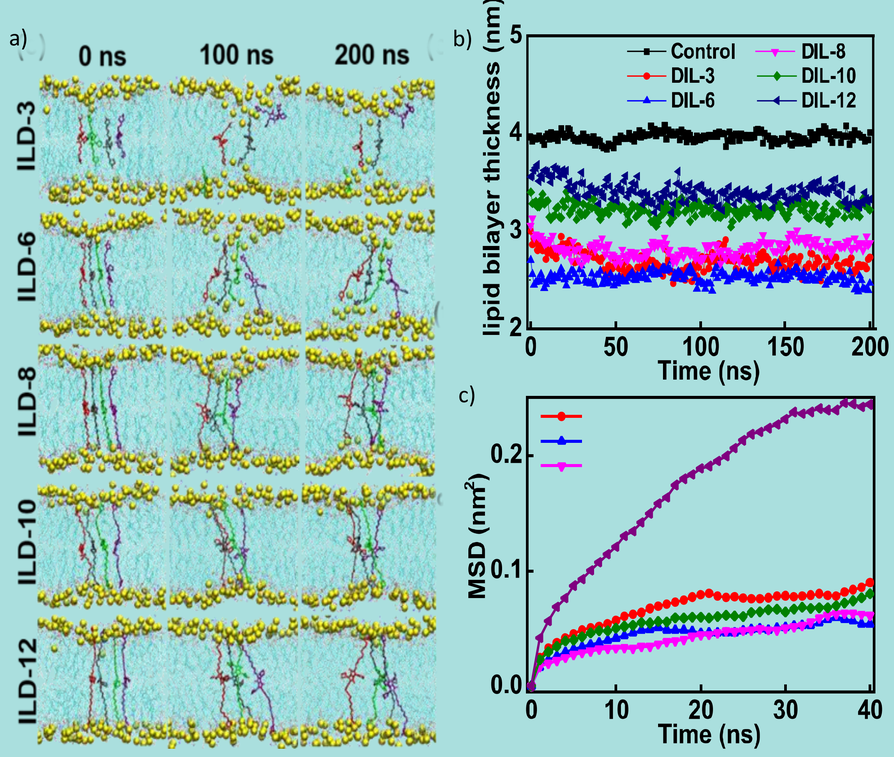


Figure 2. (a) Sequential snapshots of MD simulations for interactions between ILDs and phospholipid bilayer. (b) Thickness of POPE/POPG bilayer treated with ILDs at different time. (c) Mean square displacement (MSD) of ILDs along the lateral dimension of POPE/POPG bilayer.

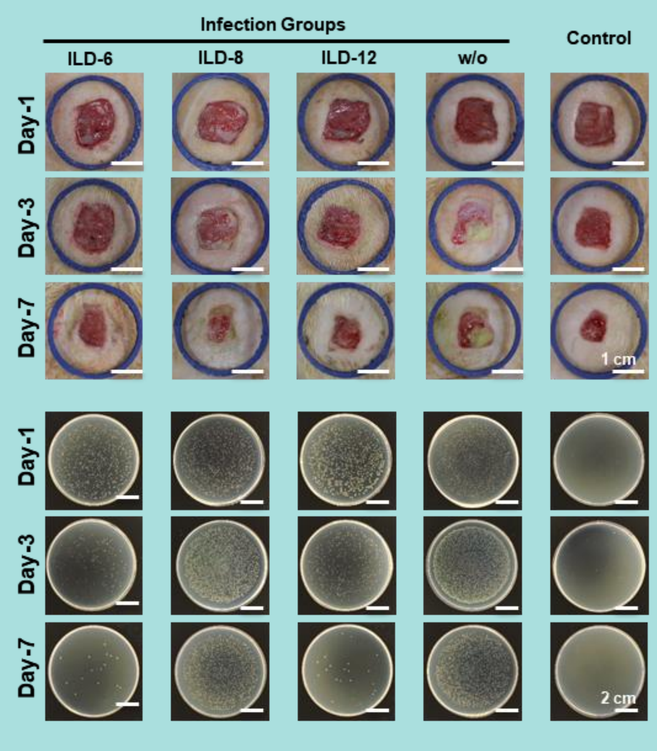


Figure 3. Wound photographs and plate counting photos in wound tissues of different groups of rats for day-1, day-3 and day-7. (PAO1 infected rat model)

*The similar structure-antibacterial activity relationships were demonstrated appropriate for another gram-negative bacteria PAO1

CONCLUSIONS

- **ILD-6 with relatively short alkyl chains could come into bacteria through membrane and showed efficient antimicrobial activity for its membrane thinning and inhibition of specific bacterial metabolic processes. ILD-12 with longest alkyl chains intercalated into bacterial membranes and effectively damaged the phospholipid by inducing the highest membrane disorder, which was equally efficient to ILD-6.**
- **ILD-8 could neither penetrate through bacterial membrane nor make effective disturbance to bacterial membrane integrity for its weakest fluctuation ability, and showed the poorest antibacterial activity of all ILDs.**
- **ILD-3 with the shortest alkyl chain length could only interacted with one single leaflet of the phospholipid bilayer, resulting in little destructive effects on bacteria membrane. ILD-1 intercalated into bacterial membrane and its disturbance effect on bacterial membrane was not as strong as ILD-12. Both ILD-3 and ILD-10 had a relatively poorer antibacterial activity than ILD-6 and ILD-12.**
- **The mechanism exploration of structure-antibacterial activity relationships of ILDs would provide useful information towards the rational design of next generation of advanced antimicrobial agents.**