Thiazolium derivative functionalized silver nanocomposites for suppressing bacterial resistance and eradicating biofilm

Xiaomei Dai, Yu Zhao, Junsheng Li, Sen Li, Ruidong Lei, Xuelei Chen, Xinge Zhang,* Chaoxing Li* 

* Key Laboratory of Functional Polymer Materials of Ministry of Education, Institute of Polymer Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China 

* Corresponding author: 

The Key Laboratory of Functional Polymer Materials, Ministry of Education, Institute of Polymer Chemistry, College of Chemistry, Nankai University, Weijin Road 94, Tianjin 300071, China 

Tel: +86-22-23501645; Fax: +86-22-23505598 

E-mail: zhangxinge@nankai.edu.cn 

E-mail: lcx@nankai.edu.cn
Materials

5-(2-Hydroxyethyl)-4-methylthiazole (97%) (ATA), acryloyl chloride, acridine orange/ethidium bromide (AO/EB), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and o-nitrophenyl-β-D-galactopyranoside (ONPG) were purchased from Tianjin Heowns Biochem Technologies LLC (Tianjin, China). 2,2’-Azobisisobutyronitrized (AIBN) and silver nitrate (AgNO₃) was gained from Alfa aesar (Beijing, China). FITC-labeled concanavalin A (ConA-FITC) was purchased from Sigma-Aldrich (Shanghai, China). NIH 3T3 cells and A549 cells were obtained from Tianjin Medical University (Tianjin, China). Phosphate buffered saline (PBS, pH 7.2) is a water-based salt solution containing disodium hydrogen phosphate (0.2 M, 72%) and sodium dihydrogen phosphate (0.2 M, 28%). Dimethyl formamide (DMF), dimethylsulfoxide (DMSO), ethyl bromide, sodium bicarbonate and the other chemical reagent were purchased from Tianjin Chemical Reagent Company (Tianjin, China) and used without purification. Escherichia coli (E. coli), S. aureus and P. aeruginosa were gained from the Department of Microbiology of Nankai University (Tianjin, China).

Synthesis of the 5-(2-hydroxyethyl acrylate)-4-methylthiazole (ATA)

ATA was prepared according to previously study with slight modification.¹ ATA (1.0 mmol), triethylamine (1.0 mmol) and 20 mL dried dichloromethane were added to an oven-dried three-necked flask. The mixture was cooled in an ice-water bath. Acryloyl chloride (1.0 mmol) was slowly added into the mixture in 30 min. The reaction was
allowed to proceed for further 24 h at room temperature. The mixture solution was
diluted with 20 mL of dichloromethane and then washed with sodium bicarbonate
solution three times (3 × 40 mL). The organic phase was dried over anhydrous
magnesium sulfate overnight. After removing the solvent by rotary evaporation, the
 crude product was purified by column chromatography to afford yellow oily liquid in
84% yield. $^1$H NMR (400 mHz, TMS, DMSO, ppm): 8.83-8.85 (1H, -S-CH=N-),
6.41-6.46 (1H, -CH$_2$=CH-), 6.11-6.17 (1H, -CH$_2$=CH-), 5.83-5.86 (1H, -CH$_2$=CH-),
4.24-4.30 (2H, -O-CH$_2$-CH$_2$-), 3.10-3.17 (2H, -O-CH$_2$-CH$_2$-), 2.30-2.36 (3H, -CH$_3$).

Preparation of poly(5-(2-hydroxyethyl acrylate)-4-methylthiazole) by
conventional free radical polymerization

A relevant monomer ATA, AIBN and the corresponding amount of anhydrous DMSO
were mixed in a 10 mL polymerization tube. The tube was sealed under high vacuum
after three freeze-evacuate-thaw cycles, and then the tube was placed in a preheated
oil bath at 70 °C with continuous stirring. After polymerization for 24 h, the reaction
mixture was cooled to 0 °C, and then diluted with DMSO. The mixture was
precipitated into 100 mL of distilled water, which was stirred vigorously, then
collected by filtration and dried under a vacuum oven at 25 °C overnight. PATA was
obtained as a yellow solid. $^1$H NMR (400 mHz, TMS, DMSO, ppm): 8.83-8.85 (1H, -
S-CH=N-), 4.24-4.30 (2H, -O-CH$_2$-CH$_2$-), 3.10-3.17 (2H, -O-CH$_2$-CH$_2$-), 2.30-2.36
(3H, -CH$_3$).

Quaternization of PATA
In this study all the purified copolymers were quaternized by redissolving in either anhydrous DMF (10 mL). An excess of ethyl bromide (butyl bromide or hexane bromide) was added and the solution was sealed under nitrogen and heated at 70 °C for 72 h. The reaction was monitored by $^1$H-NMR. After cooling to 25 °C, the product was purified by dialysis against distilled water for 3 days. After freeze-drying under vacuum, the desired PATA-Cn was obtained. The degree of substitution was obtained by elemental analysis. $^1$H NMR (400 mHz, TMS, DMSO, ppm): 8.75-8.83 (1H, \(-S-CH=\text{N}\)-), 4.52-4.48 (-N-\text{CH}2-\text{CH}2-), 4.24-4.30 (2H, \(-\text{O-CH}2-\text{CH}2-\)), 3.10-3.17 (2H, \(-\text{O-CH}2-\text{CH}2-\)), 2.30-2.36 (3H, \(-\text{CH}3\)), 0.89-1.45 (-\text{CH}2-\text{CH}2-\text{CH}3).

The minimal inhibitory concentration (MIC) of the antibacterial agent that inhibited visible growth of the microorganism in a broth dilution susceptibility test which was determined according to the Clinical and Laboratory Standards Institute with a modified method. The lowest concentration (MIC value) was the one at which there was no turbidity greater than the faint turbidity. Each assay was carried out in triplicate. This assay was used to investigate the viability of bacteria after being treated with antibacterial agents. The live bacteria will proliferate and increase the turbidity of the mixture. The value of optical density at 600 nm (OD$_{600}$) was used to measure the turbidity of the mixture to evaluate the antibacterial activity.

**Statistical Analysis**

All data were displayed as a mean ± standard deviation of three or five different experiments, and compared via Kruskal-Wallis one-way analysis of variance (ANOVA), where $p < 0.05$ (significant difference).
Table S1 The physical and biology properties of PATA-Cn.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mn</th>
<th>PDI</th>
<th>Degree of substitution (%)</th>
<th>MIC (μg/mL)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>P. aeruginosa</td>
<td>S. aureus</td>
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<tr>
<td>PATA1-C4</td>
<td>5.3</td>
<td>1.50</td>
<td>80.7</td>
<td>250 ± 25*</td>
<td>250 ± 23*</td>
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<td>PATA2-C4</td>
<td>7.1</td>
<td>1.59</td>
<td>81.3</td>
<td>62.5 ± 15*</td>
<td>62.5 ± 14*</td>
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<tr>
<td>PATA3-C4</td>
<td>9.0</td>
<td>1.50</td>
<td>79.5</td>
<td>31.3 ± 17</td>
<td>31.3 ± 11</td>
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</tr>
<tr>
<td>PATA4-C4</td>
<td>11.0</td>
<td>1.48</td>
<td>81.5</td>
<td>125 ± 19*</td>
<td>125 ± 21*</td>
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<tr>
<td>PATA3-C2</td>
<td>9.0</td>
<td>1.50</td>
<td>82.2</td>
<td>125 ± 20*</td>
<td>125 ± 22*</td>
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<tr>
<td>PATA3-C6</td>
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<td>1.50</td>
<td>79.5</td>
<td>250 ± 26*</td>
<td>125 ± 24*</td>
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</tr>
</tbody>
</table>

Number-average molecular weight (Mn) and polydispersity index (PDI). Asterisks indicate significant differences between PATA3-C4 and the other material in each column: *p < 0.05 (extremely significant).
Fig. S1 TEM images of PATA-C4@AgNPs with different mass ratios of PATA-C4 to AgNPs: (A) 10:1; (B) 15:1; (C) 20:1 and (D) 30:1.

Fig. S2 The size distribution of PATA-C4@AgNPs in aqueous solution.
Fig. S3 XRD pattern of PATA-C4@AgNPs (5:1).

Fig. S4 XPS spectra of the PATA-C4@AgNPs: (A) survey spectra, and (B) Ag 3d region XPS spectrum.