

Supporting Information

5 **Metallic Nanoparticles Bioassay for *Enterobacter cloacae* P99 β -
Lactamase Activity and Inhibitor Screening**

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Identification of Class C *E. cloacae* P99 β -lactamase. The purity and isoform components of Class C *E. cloacae* P99 β -lactamase was analyzed by SDS-PAGE. As shown in Figure S1, the purified *E. cloacae* P99 β -lactamase exhibited one band with molecular weight of 39 KDa which indicated the higher molecular weight than that of transformed TEM-1 β -lactamase (29 KDa). The result is consistent with the previous report.¹

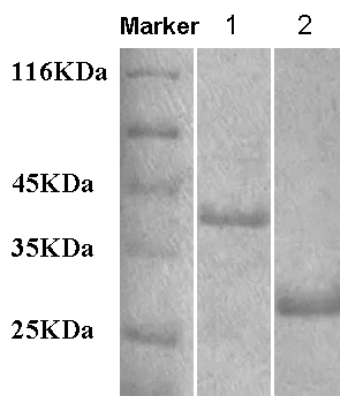
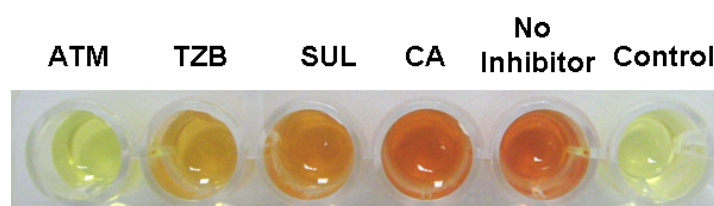


Figure S1. SDS-PAGE analysis of *E. cloacae* P99 class C Bla (Lane 1, 12 μ M) with molecular weight of 39 KDa and transformed TEM-1 class A Bla (Lane 2, 12 μ M) with molecular weight of 29 KDa.
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Colorimetric inhibition assay by using Nitrocefin: Class C *E. cloacae* P99 Bla was initially incubated with different inhibitors in phosphate buffer saline (PBS buffer, pH 7.4) for 10 min and subsequently β -lactam substrate was added for additional 20 min incubation. Then, the aliquot of 15 above mixture solution was transferred into nitrocefin solution affording 5 μ M of substrate, 5 μ M of inhibitor and 3 nM of *E. cloacae* P99 Bla.



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Figure S2. Colorimetric inhibition assay for *E. cloacae* P99 Bla in 96-well microplate with four kinds of inhibitors.

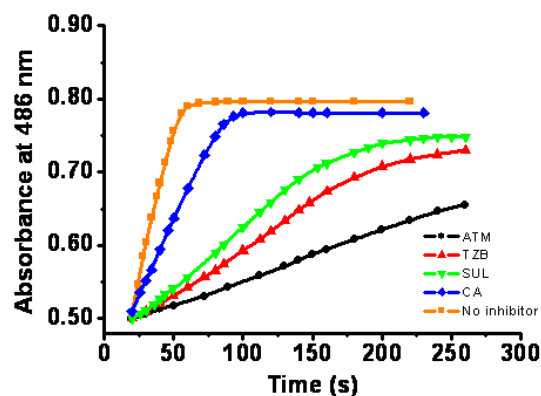
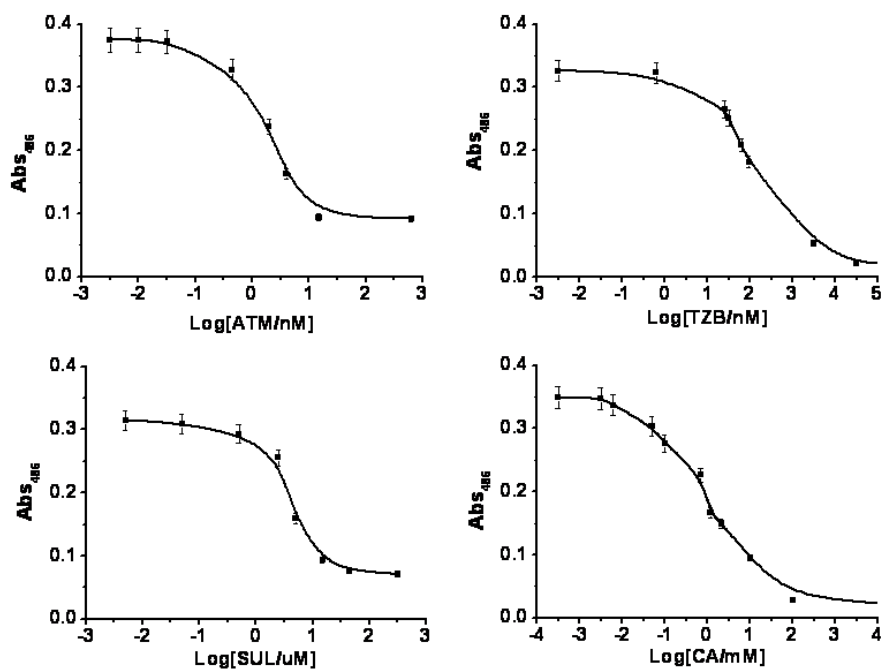


Figure S3. Absorbance change of nitrocefin at 486nm as a function of time in the presence of *E. cloacae* P99 Bla inhibitors.

5 As shown in Figure S4, IC_{50} values of ATM, TZB, SUL and CA was evaluated with nitrocefin to be 3.0 nM, 165.9 nM, 5.5 μ M and 1.12 mM, respectively. This result is consistent with the IC_{50} values recognized by using AgNPs and AuNPs.



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Figure S4. IC_{50} graph of ATM, TZB, SUL and CA by using absorbance change of nitrocefin at 486 nm.

Reference:

- 15 (1) Bush, K.; Jacoby, G. A.; Medeiros, A. A. *Antimicrob. Agents Chemother.* **1995**, *39*, 1211-1233.