Supporting Information

N-heterocyclic Molecules-capped Gold Nanoparticles as Effective Antibiotics against Multi-drug Resistant Bacteria

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Yan Feng and Wenwen Chen have equal contribution to the paper.

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3. Characterization.
4. Antimicrobial test.
5. Cytotoxicity assay of gold NPs.
6. Superthin slices preparation for SEM and TEM observation.
7. Calculation of Numbers of Au Atoms and Pyrimidines per NP

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2. **Figure S2.** TEM images of ATT-Au NPs, AHMT-Au NPs, MI-Au NPs, MTM-Au NPs, AMP-Au NPs and AMBT-Au NPs with the initial molar ratio (1:1) of N-heterocyclic molecules to $HAuCl_4$
3. **Figure S3.** The diameter distribution of ATT-Au NPs, AHMT-Au NPs, MI-Au NPs, MTM-Au NPs, AMP-Au NPs and AMBT-Au NPs with different initial molar ratio (1:1 and 10:1) of N-heterocyclic molecules to $HAuCl_4$
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heterocyclic molecules to Au NPs and surface charges in water at 25°C on *E.coli* and MDR *Ecoli.*

Experimental Section

1. **Chemicals and materials.** $HAuCl_4 \cdot 3H_2O$ (99.99%) was from Jinke Chemical Co., Ltd., China. 3-Amino-1,2,4-triazole-5-thiol (ATT), 4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole (AHMT), 2-Mercaptoimidazloe (MI), Methimazole (MTM), 2-Amino-6-mercaptopurine (AMP) and 6-Amino-2-mercaptobenzothiazole (AMBT) were from Sigma. Standard strains of *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphyloccocusaureus aureus* (ATCC 6538P) were from China General Microbiological Culture Collection Center (Institute of Microbiology of Chinese Academy of Sciences) and a standard strain of Klebsiella peneumoniae (ATCC 13883) was from Agricultural Culture Collection of China. The clinical isolates, MDR *Escherichia coli*, MDR *Pseudomonas aeruginosa* and *S. aureus* MRSA were from local hospitals in China. Human umbilical vein endothelial cells (HUVEC) were a gift from Prof. Xiyun Yan at Institute of Biophysics, Chinese Academy of Sciences.
2. **Synthesis of different heterocyclic molecules-capped gold NPs.** We stirred the mixture of heterocyclic small molecule (0.1 mmol, dissolved in 10 mL methanol, 200μL absolute acetic acid and 40 mg of Tween 80) and $HAuCl_4 \cdot 3H_2O$ (0.1 mmol, dissolved in 20 mL methanol) for 10 min in the ice-water bath, added $NaBH_4$ (0.3mmol, freshly dissolved in 5 mL methanol) dropwise with vigorous stirring. We decreased the stirring speed and kept stirring the solution for another hour in the ice-water bath. We removed methanol in vacuum at 40°C, added an appropriate volume of deionized water into the residue, dialyzed (14 kDa MW cut off, Millipore) the solution for 48 h with deionized water, diluted it with deionized water, sterilized it through a 0.22 μm filter (Millipore) and stored it at 4°C for use. Synthesis of other molar ratios of samples is similar, except we changed the concentration of heterocyclic molecules from 0.1 mmol to 1 mmol.
3. **Characterization.** The concentrations of gold NPs and element of S were determined with inductively coupled plasma analysis (ICP, Elmer Optima 5300DV). We observed the morphologies of NPs using Tecnai G_2 20 ST TEM from the American FEI company. And we tested zeta potential values of gold NPs with Zetasizer Nano ZS (Malvern Instruments).
4. **Antimicrobial test.** We tested the antimicrobial activities of synthesized NPs using microtitre broth dilution method which determines the minimum inhibitory concentration (MIC) leading to the inhibition of microbial growth (NCCLS M7-A6, 2003). Disposable Corning 96-well plates were used for the tests. We diluted

the synthesized NPs solutions 2-128 times with 100 μL of nutrient broth inoculated with the tested microbe at a concentration of 2×10^4 CFU/mL to 2×10^5 CFU/mL. The minimum inhibitory concentration (MIC) was observed after 24 h of incubation at 37 °C as the MIC of the tested substance that inhibited the growth of the microbial strain.

5. **Cytotoxicity assay of gold NPs.** Human umbilical vein endothelial cell (HUVEC) lines were cultured in the DMEM mixing medium (100 ml of the DMEM mixing medium includes 83 mL of Gibco DMEM, 15 mL of fetal bovine serum, 29.2 mg of Glutamine, 10,000 units of Penicillin and 10,000 units of Streptomycin). We incubated 1×10^4 cells per well of HUVEC cell lines in 96-well plates with 0, 10, 20, 50, 100 μg mL^{-1} of Au NPs in 200 μL of DMEM medium for 24 h. After discarding the supernatant, we added 10% (v/v) of the CCK-Kit solution in DMEM and incubated the sample at 37 °C for 2 h. The absorbance at 450 nm (with reference wavelength of 650 nm) was determined by Tecan infinite 200 multimode micro plate readers.
6. **Superthin slices preparation for SEM and TEM observation.** After six hours of treatment of *E.coli* and *S. aureus* with MI-Au at 10 μg/mL and 25μg/mL on shaking bed at 200 rpm 37 °C, we chose 10 μg/mL for *E.coli* and 25μg/mL for *S. aureus*, because at this concentration, we can most easily obtain bacterial cell samples and observe the changes of cell walls with SEM and TEM. Samples underwent fixing with 2.5% glutaraldehyde and 30%, 50%, 70%, 80%, 90%, 100% ethanol dehydration for SEM. TEM samples underwent fixing with 2.5% glutaraldehyde and 0.1% osmic acid, dehydrating with graded ethanol, and further cutting superthin slices and staining with 2% uranyl acetate and 0.2% lead citrate.
7. **Calculation of Numbers of Au Atoms and Pyrimidines per NP.** We calculate the number of gold atoms and ligands per NP using reported methods.[1] Gold atoms are close-packed and form an fcc (Face Centred Cubic) structure in NPs. The number of gold atoms, $N_{Au} = (V_{NP} \times APF)/\, V_{Au} = (4\pi {r_{NP}}^3/3 \times 0.7405)/(4\pi {r_{Au}}^3/3) = (d_{NP}/2\ nm)^3 \times 31$

   where V_{NP} is the volume of asphere gold NP, $V_{Au}$is the volume of a gold atom, APF is atomic packing factor, r_{NP} and d_{NP} are the radius and the diameter of a sphere gold NP, and r_{Au} is the radius of a gold atom. The number Ns of thiolate ligands per NP, $N_S = N_{Au} \times X$, X is the molar ratio of S to Au in NPs.

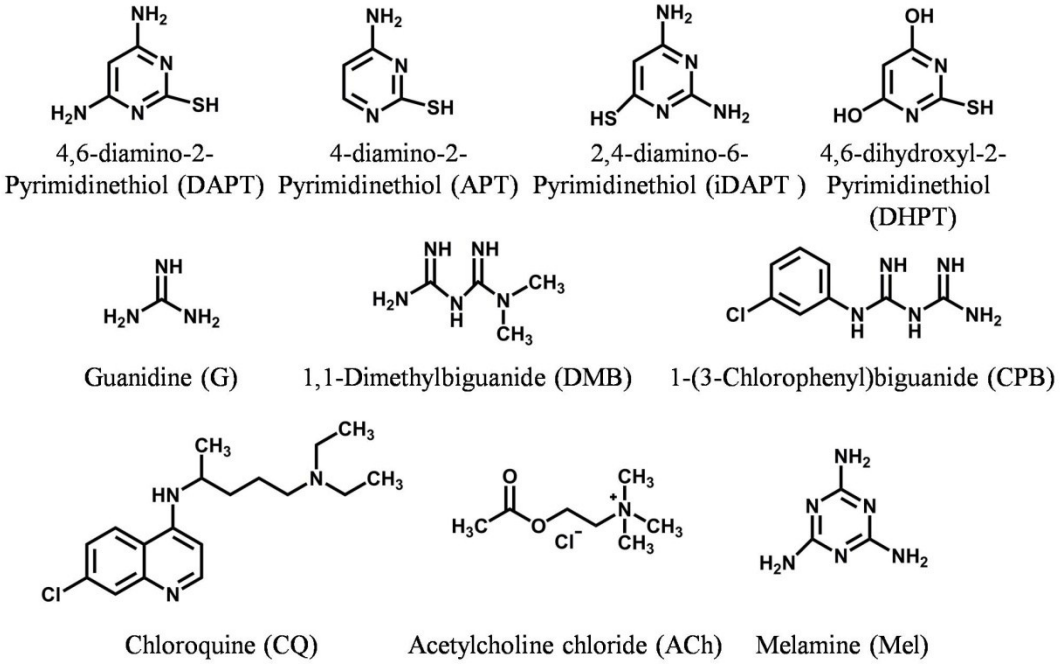

Figure S1. The structure of our group previous screened molecules.

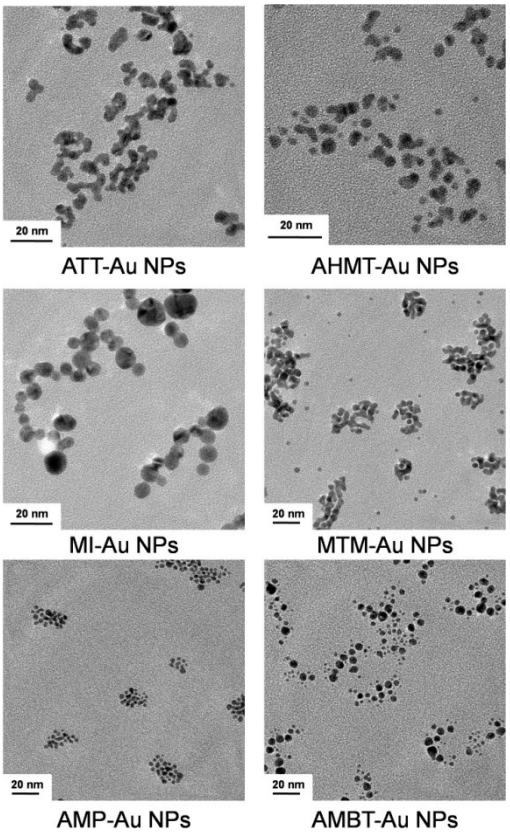

Figure S2. TEM images of ATT-Au NPs, AHMT-Au NPs, MI-Au NPs, MTM-Au NPs, AMP-Au NPs and AMBT-Au NPs with the initial molar ratio (1:1) of N-heterocyclic molecules to $HAuCl_4$.

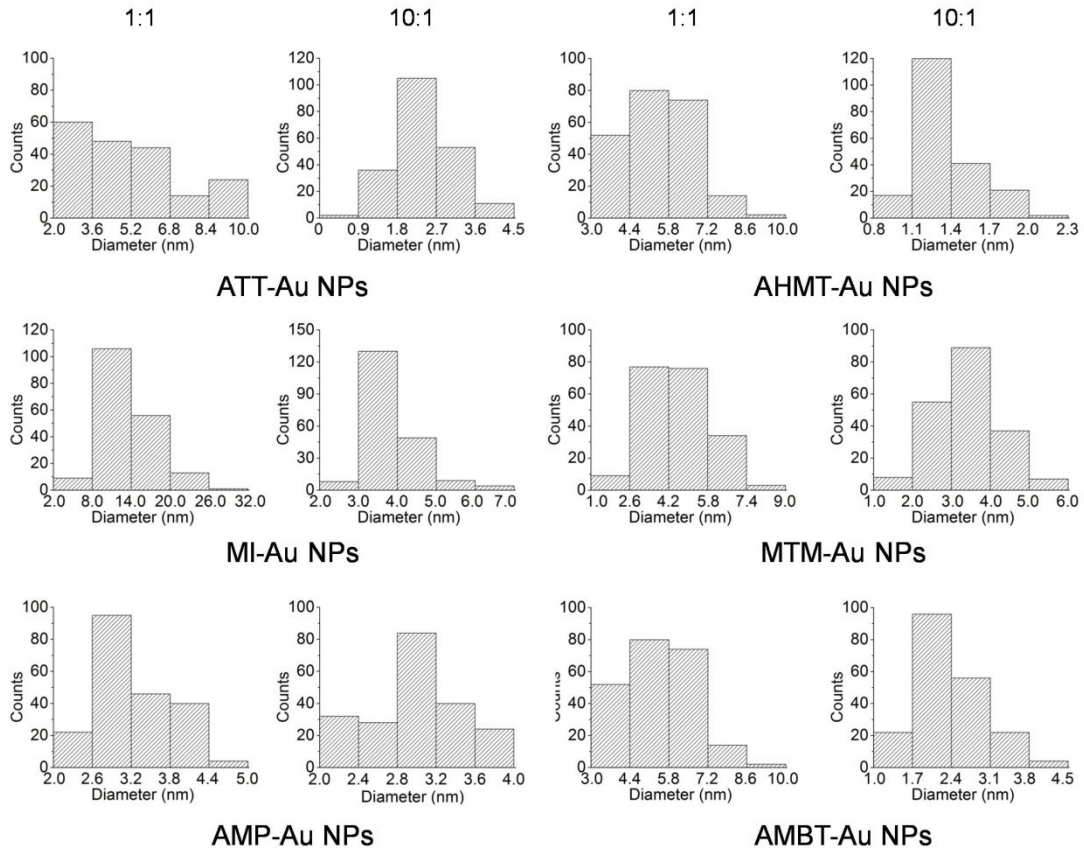

Figure S3. The diameter distribution of ATT-Au NPs, AHMT-Au NPs, MI-Au NPs, MTM-Au NPs, AMP-Au NPs and AMBT-Au NPs with different initial molar ratio (1:1 and 10:1) of N-heterocyclic molecules to $HAuCl_4$.

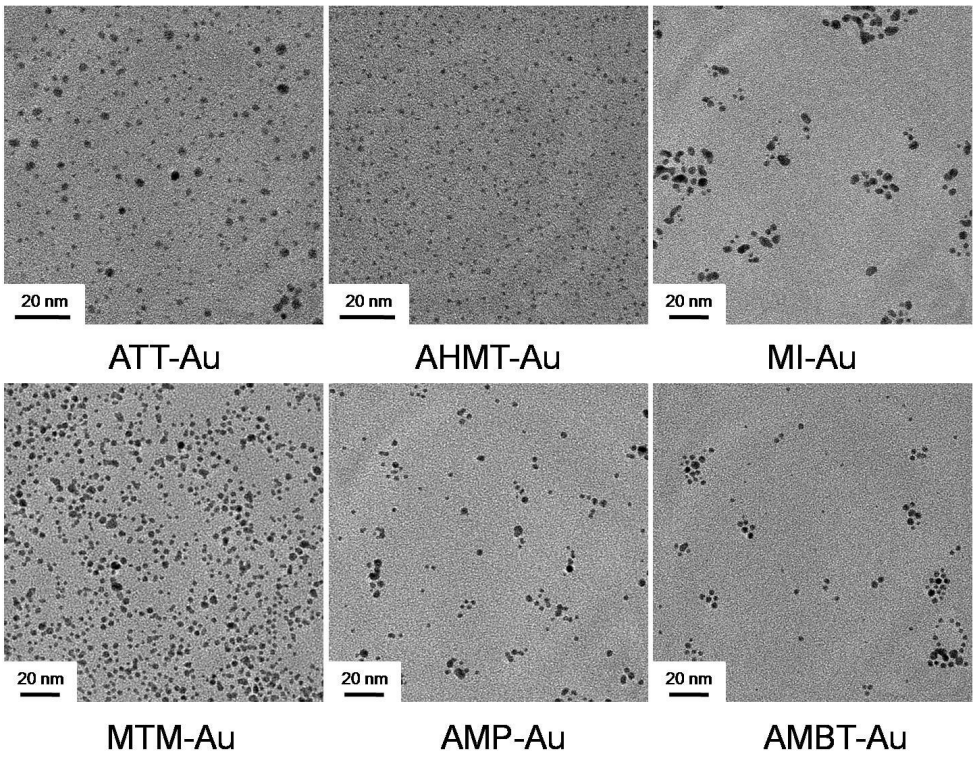

Figure S4. TEM images of ATT-Au NPs, AHMT-Au NPs, MI-Au NPs, MTM-Au

NPs, AMP-Au NPs and AMBT-Au NPs with the initial molar ratio (10:1) of N-heterocyclic molecules to $HAuCl_4$ after one year.

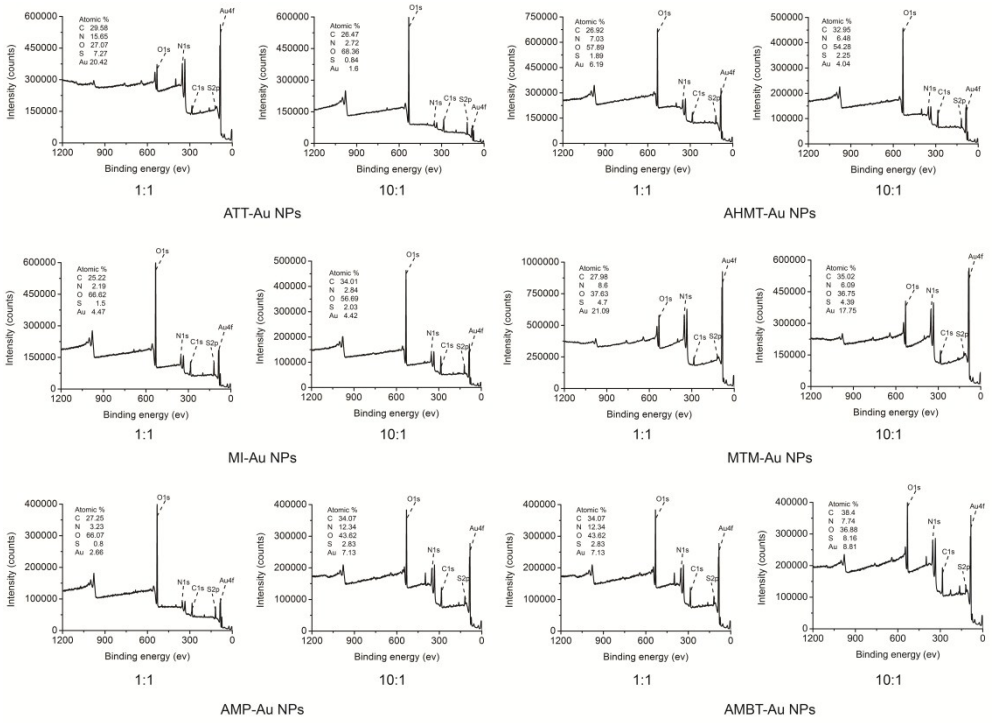

Figure S5. XPS characterization of Au NPs coated with different N-heterocyclic molecules.

Table S1. Components of Au NPs in water at 25 ^{o}C.

	Molar ratio of pyrimidine to Au in NPs	Au atoms per NP	N-heterocyclicperNPs
ATT:$HAuCl_4$=1	0.36	15872	5714
ATT:$HAuCl_4$=10	0.53	461	245
AHMT:$HAuCl_4$=1	0.31	4854	1505
AHMT:$HAuCl_4$=10	0.56	85	48
MI:$HAuCl_4$=1	0.34	70171	23858
MI:$HAuCl_4$=10	0.46	1674	770
MTM:$HAuCl_4$=1	0.22	6048	1331
MTM:$HAuCl_4$=10	0.25	1240	310
AMP:$HAuCl_4$=1	0.30	1124	337
AMP:$HAuCl_4$=10	0.40	1114	334
AMBT:$HAuCl_4$=1	0.4	4334	1733
AMBT:$HAuCl_4$=10	0.92	445	409

We calculate the number of gold atoms and ligands per NP using reported methods.[1]

Table S2. Comparison of surface charges in water at 25°Cand the antibacterial activity on *E.coli* and *MDR E.coli* of Au NPs with different molar ratio of heterocyclic molecules to $HAuCl_4$.

	Zeta Potential (mV)	Minimum Inhibition Concentrations (μg/mL)	
		E.coli	MDR *E.coli*
ATT:$HAuCl_4$=1	20.4±14.4	32	32
ATT:$HAuCl_4$=10	2.43±7.72	4	4
AHMT:$HAuCl_4$=1	4.63±13.0	16	16
AHMT:$HAuCl_4$=10	2.11±8.75	4	4
MI:$HAuCl_4$=1	8.22±9.79	>128	>128
MI:$HAuCl_4$=10	2.03±11.2	4	4
MTM:$HAuCl_4$=1	2.52±10.3	>128	>128
MTM:$HAuCl_4$=10	11.5±9.45	16	16
AMP:$HAuCl_4$=1	22.4±3.78	8	8
AMP:$HAuCl_4$=10	26.9±8.34	8	8
AMBT:$HAuCl_4$=1	7.4±3.78	16	16
AMBT:$HAuCl_4$=10	18.5±3.05	8	8

References

1.Y. Y. Zhao, Y. Tian, Y. Cui, W. W. Liu, W. S. Ma and X. Y. Jiang, *J Am Chem Soc*, 2010, **132**, 12349-12356.