Supporting Text

Expression and Purification of *Escherichia coli* FtsZ. Untagged *E. coli* FtsZ was expressed from pET-3Z+ in BL21(λDE3) cells and purified by ammonium sulfate fractionation followed by ion-exchange chromatography on a Mono-Q HR 5/5 column (Amersham Pharmacia-Pharmacia), as described (1, 2). Alternatively, *E. coli* FtsZ was purified using a two-step ammonium sulfate fractionation (3).

Expression and Purification of *Mycobacterium tuberculosis* FtsZ. *M. tuberculosis* ftsZ was amplified by PCR from H37Rv-derived cosmid clone Y270 (4; a kind gift of K. Eiglmeier and S. T. Cole, both from Institut Pasteur, Paris) using primers 5'

GAGCATATGACCCCCCGCACAAG (forward primer; *NdeI* site in bold) and 5'GAGGGATCCCTCAGCGGCGCATGAAAG (reverse primer; *BamHI* site in bold). The 1,140-bp ftsZ fragment was cloned into the *NdeI* and *BamHI* sites of pET-16b (Novagen) to obtain pRM4, which was sequenced to rule out any mutational error arising during PCR. FtsZ, tagged at its N terminus with His<sub>6</sub>, was expressed from pRM4 in BL21(λDE3)/pLysS cells in LB medium at 37°C. Overnight-grown cells were diluted 1:250 in 1 liter of LB medium and grown to a cell density of Klett ≈60. His<sub>6</sub>-FtsZ was induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 5 h. Cells were harvested by centrifugation, washed with cold PBS (pH 7.4), and resuspended in PBS. Cell extracts were prepared by three cycles of freeze-thaw, followed by brief sonication. Soluble and particulate fractions were separated by centrifuging cell extracts at 45,000 rpm in a 60.2 Ti rotor (Beckman) for 60 min at 4°C. His<sub>6</sub>-FtsZ resided predominantly in the soluble
fraction. To purify the protein, a 2-ml His-Trap Ni\textsuperscript{2+}-chelate column (Amersham Pharmacia) was equilibrated with 10 ml of buffer A (20 mM sodium phosphate, pH 7.0/500 mM NaCl/10 mM imidazole). The soluble protein sample (≈14 mg of protein) was loaded onto the column, which was washed with 15 ml of buffer B (buffer A modified to contain imidazole at 100 mM). His\textsubscript{6}-FtsZ was eluted with 5 ml of buffer C (buffer A modified to contain imidazole at 300 mM). The peak fractions were pooled, dialyzed extensively against buffer D (50 mM Tris•HCl, pH 7.5/5 mM MgCl\textsubscript{2}/0.1 mM EDTA/10% glycerol), and stored in buffer D (•2 mg/ml protein concentration) at –70°C. The purity of His\textsubscript{6}-FtsZ was >98% as judged by overloading the protein on a 12% SDS-polyacrylamide gel and staining it with Coomassie brilliant blue R250.

**GTPase Assays.** Assays were performed using 2 \(\mu\)M *E. coli* FtsZ or 12.5 \(\mu\)M *M. tuberculosis* FtsZ in buffer E (50 mM 4-morpholinepropanesulfonic acid, pH 6.5/50 mM KCl/5 mM MgCl\textsubscript{2}) containing 0.1% Tween-20. To assess dose-dependent inhibition of FtsZ GTPase, the compounds (in DMSO) were added to the desired final concentrations such that the DMSO concentration in the samples was ≈3%. The control tubes received 3% DMSO alone. The charcoal-based radioactive assay was adapted from Lee *et al.* (5). After a 5-min preincubation of FtsZ with Zantrins at 30°C, the reactions were initiated by adding 1 mM \([\gamma^{32}\mathrm{P}]\) GTP (200-500 cpm/pmole). At specific time points, 6-\(\mu\)l aliquots were withdrawn and added to 600 \(\mu\)l of 5% (wt/vol) activated charcoal in 50 mM NaH\textsubscript{2}PO\textsubscript{4}. Samples were immediately vortexed and placed on ice. All samples were centrifuged at 14,000 rpm (Eppendorf microfuge 5415, Brinkmann) for 20 min to pellet the charcoal, and 80-\(\mu\)l supernatants were withdrawn for quantification of \(^{32}\mathrm{P}\).
scintillation counting. The turnover rate of FtsZ GTPase was calculated by using values from the linear phase of GTP hydrolysis, and percent inhibition was determined by comparing rates of hydrolysis in the presence or absence of inhibitors.

In the nonradioactive malachite green-phosphomolybdate assay (6), FtsZ was preincubated with or without varying concentrations of Zantrins in buffer E as above, and the reactions were initiated by adding 1 mM GTP. The control tubes received an appropriate amount of DMSO. Aliquots were withdrawn at 10-min intervals and quenched with 20 mM EDTA before color development with the malachite green solution [2 volumes malachite green (0.8 mg/ml)/1 volume polyvinyl alcohol (23.2 mg/ml)/1 volume ammonium molybdate (57.2 mg/ml in 2H₂O:3HCl)/2 volumes H₂O]. One hundred twenty microliters of malachite green solution and 30 µl of 34% (wt/vol) sodium citrate were added to 5-µl reaction aliquots, the samples were incubated for 30 min at room temperature, and the absorbance was read at 650 nm. A standard dilution series of known phosphate concentrations was included in each experiment to quantify the extent of GTP hydrolysis.

**Determination of Minimum Inhibitory Concentrations (MICs).** All bacterial organisms shown in Table 2, except *Streptococcus pneumoniae* and *Clostridium perfringens*, were grown in LB medium. The organisms were cultured at 37°C, except *E. coli* strains that were grown at 30°C. *S. pneumoniae* TIGR4 strain was grown in 5% CO₂ atmosphere as standing cultures in Todd-Hewitt broth supplemented with 5% yeast extract and 5 µg/ml Oxyrase. *C. perfringens* strain 13 was grown anaerobically (5% CO₂,
10% H₂, and 85% N₂ environment) as standing cultures in a broth containing 3% tryptone, 2% yeast extract, 1% glucose, and 0.1% thioglycollate (pH ≈ 7.4). To determine MICs, fresh cultures were inoculated at an initial cell density of ≈1-2 × 10⁵ cells/ml and the compounds were added at 80 µM. A 2-fold serial dilution series of culture tubes was set up in duplicate. All organisms, except *S. pneumoniae* and *C. perfringens*, were grown on a rotary wheel for 14-16 h. The control tubes received an identical amount of DMSO as that present in tubes containing the highest inhibitor concentration (typically between 1–2%). Bacterial growth was assessed visually, and the lowest inhibitor concentration that caused >99% growth inhibition was designated the MIC.