Supporting Information

Structural Flexibility of an Inhibitor Overcomes Drug Resistance Mutations in *Staphylococcus aureus* FtsZ

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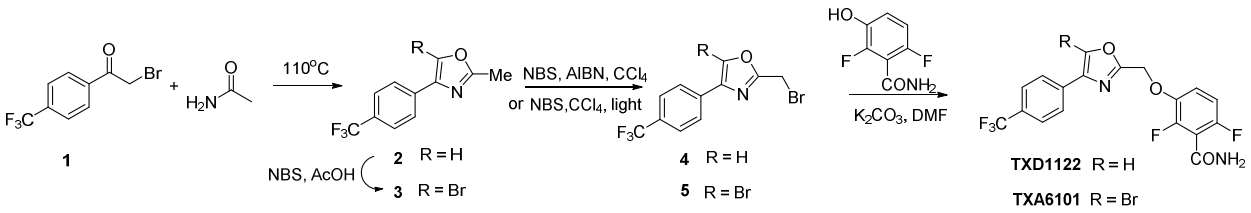
SUPPLEMENTAL EXPERIMENTAL METHODS

Synthesis of TXD1122 and TXA6101.

TXD1122 and TXA6101were synthesized using an approach similar to that previously reported for TXA6101 [1].



*Preparation of 2-methyl-4-(4-(trifluoromethyl)phenyl)oxazole (**2**):* A mixture of 4-trifluoromethylphenacyl bromide (1.14 g, 4.28 mmol) and acetamide (550 mg, 8.56 mmol) was heated to 110°C for 2 hours. When the reaction was complete, water was added and the mixture was washed with ethyl acetate (3 x 100 mL). The combined organic layers were washed with water, dried with Na_2SO_4, filtered and concentrated. The crude mixture was purified with an ISCO chromatograph on silica using 5% EtOAC/hexane to give the desired product as off white solid, yield 700 mg, 59%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.88 (s, 1H), 7.81 (d, *J*= 8.1 Hz, 2H), 7.63 (d, *J* = 8.4 Hz, 2H), 2.52 (s, 3H).

*Preparation of 5-bromo-2-methyl-4-(4-(trifluoromethyl)phenyl)oxazole (**3**):* To a solution of 2-methyl-4-(4-(trifluoromethyl)phenyl)oxazole (294 mg, 1.3 mmol) in AcOH (5.0 mL) was added NBS (230 mg, 1.3 mmol) and the resulting mixture was stirred at room temperature for 3 hours. After completion, the reaction the mixture was poured onto crushed ice and the product was extracted with ethyl acetate (3x). The combined organic layers were washed with saturated $NAHCO_3$ solution and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified with an ISCO chromatograph on silica to give the product as white solid,

yield 345 mg, 87%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.06 (d, *J* = 9.0 Hz, 2H), 7.68 (d, *J* = 9.0 Hz, 2H), 2.53 (s, 3H).

*Preparation of 2-(bromomethyl)-4-(4-(trifluoromethyl)phenyl)oxazole (**4**):* To a solution of 2-methyl-4-(4-(trifluoromethyl)phenyl)oxazole (200 mg, 0.72 mmol) in CCl_4 (3.5 mL) was added NBS (310 mg, 1.44 mmol). The resulting mixture was exposed to a lamp for 2 hours. After cooling to room temperature, the mixture was filtered, concentrated under reduced pressure and purified with ISCO chromatograph on silica to give the desired product as a mixture with the dibromo product and recovered starting material, which was used as a mixture for the next step.

*Preparation of 5-bromo-2-(bromomethyl)-4-(4-(trifluoromethyl)phenyl)oxazole (**5**):* To a solution of 2-methyl-4-(4-(trifluoromethyl)phenyl)oxazole (340 mg, 1.11 mmol) in CCl_4 (8.0mL) was added NBS (205 mg, 1.15 mmol) and AIBN (28 mg, 0.17 mmol). The resulting mixture was heated to 80 °C for 3 hours. The mixture was filtered, concentrated under reduced pressure and purified with ISCO chromatograph on silica to give the pure product along with dibromo product and recovered starting material, yield 110 mg, 26%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (d, *J* = 8.4 Hz, 2H), 7.69 (d, *J* = 8.4 Hz, 2H), 4.48 (s, 2H).

*Preparation of 2,6-difluoro-3-((4-(4-(trifluoromethyl)phenyl)oxazol-2-yl)methoxy)benzamide (**TXD1122**):* A 25-mL round bottom flask equipped with a magnetic stirrer, a condenser and a nitrogen in/outlet adapter was charged with impure bromomethyl intermediate **4** (100 mg), DMF (4.0 mL), K_2CO_3 (69 mg, 0.5 mmol), and phenol (60 mg, 0.35 mmol). The reaction mixture was stirred at 50 °C for 4 hours. The reaction mixture was diluted with EtOAc, washed with water, 10% LiCl, brine, dried over Na_2SO_4, concentrated and purified on silica gel. Elution with 70% EtOAc/hexanes afforded the title compound as white solid, yield 15 mg (less than 20 % yield). ^{1}H NMR (DMSO-d6, 300 MHz) δ 8.83 (s, 1H), 8.11 (s, 1H), 7.99-

7.96 (m, 3H), 7.83-7.77 (m, 3H), 7.36 (m, 1H), 7.10 (m, 1H), 5.38 (s, 2H). HRMS (ESI) calculated for $C_{18}H_{11}F_5N_2O_3$ $(M+H)^+$ 390.0763, found 390.0763.

*Preparation of 3-((5-bromo-4-(4-(trifluoromethyl)phenyl)oxazol-2-yl)methoxy)-2,6-difluorobenzamide (**TXA6101**):* A 25-mL round bottom flask equipped with a magnetic stirrer, a condenser and a nitrogen in/outlet adapter was charged with bromomethyl intermediate **5** (70 mg, 0.181 mmol), DMF (2.5 mL), K_2CO_3 (50 mg, 0.36 mmol), and phenol (60 mg, 0.346 mmol). The reaction mixture was stirred at 50 °C for 4 hours. The reaction mixture was diluted with EtOAc, washed with water, 10% LiCl, brine, dried over Na_2SO_4, concentrated, and purified on silica gel. Elution with 70% EtOAc/hexanes afforded the desired compound as white solid, yield 50mg, 58%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (d, J = 8.4 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 7.23 (m, 1H), 6.91 (m, 1H), 5.96 (bs, 2H), 5.21 (s, 2H). HRMS (ESI) calculated for $C_{18}H_{10}BrF_5N_2O_3$ $(M+H)^+$ 476.9868, found 476.9876.

Reference

(1) Stokes, N. R., Baker, N., Bennett, J. M., Chauhan, P. K., Collins, I., Davies, D. T., Gavade, M., Kumar, D., Lancett, P., Macdonald, R., Macleod, L., Mahajan, A., Mitchell, J. P., Nayal, N., Nayal, Y. N., Pitt, G. R., Singh, M., Yadav, A., Srivastava, A., Czaplewski, L. G., and Haydon, D. J. (2014) Design, Synthesis and Structure-Activity Relationships of Substituted Oxazole-Benzamide Antibacterial Inhibitors of FtsZ, *Bioorg. Med. Chem. Lett. 24*, 353–359.

SUPPLEMENTAL FIGURES

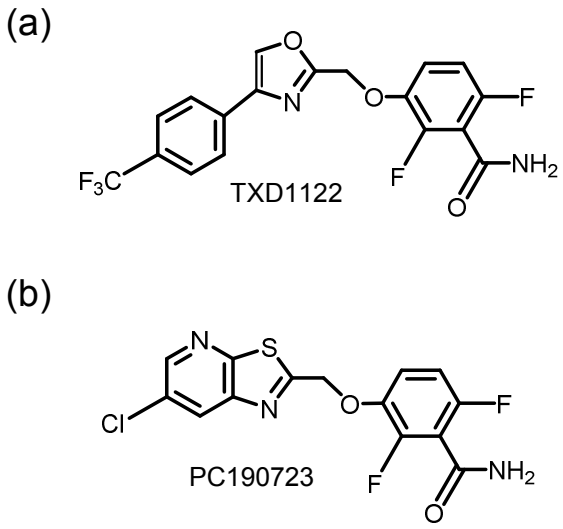


Figure S1. Chemical structures of TXD1122 (a) and PC190723 (b).

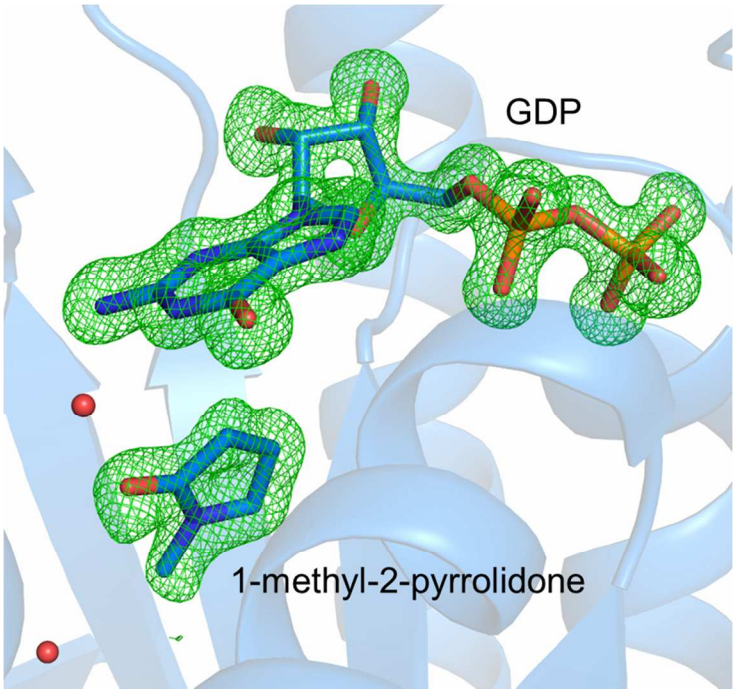


Figure S2. Expanded view of the GDP binding site of wildtype SaFtsZ in complex with TXA707, with the F_o-F_c omit map (green) being contoured at 3.0 σ. 1-methyl-2-pyrrolidone binds in an inner space near the GDP molecule.

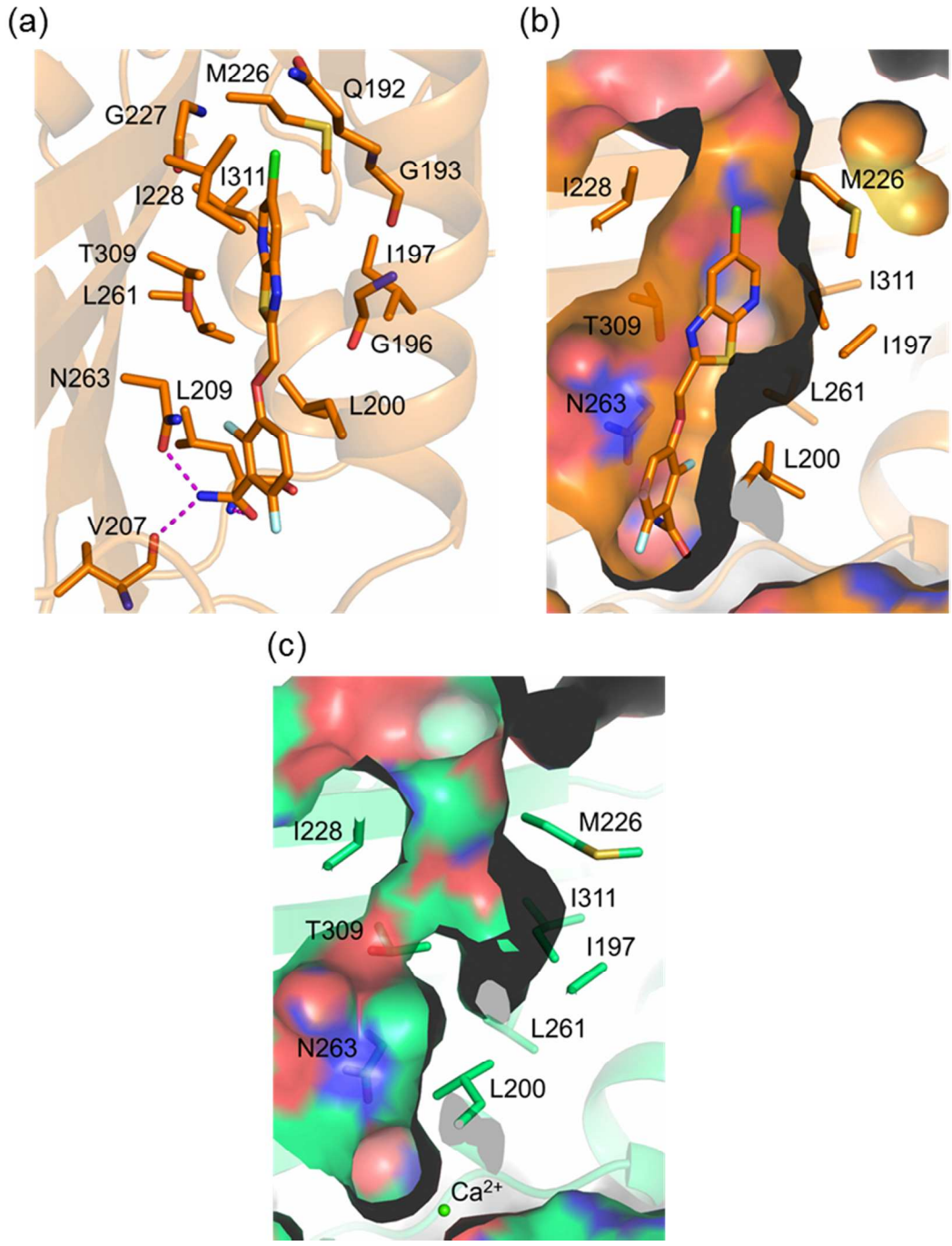


Figure S3. Previous crystal structures of wildtype SaFtsZ. (a) Binding site of PC190723 in complex with wildtype SaFtsZ (PDB entry: 4DXD), depicted as in Figure 2A. (b) Molecular surface of the PC190723 binding pocket. (c) Molecular surface of the binding pocket in inhibitor-free wildtype SaFtsZ (PDB entry: 3VOA).

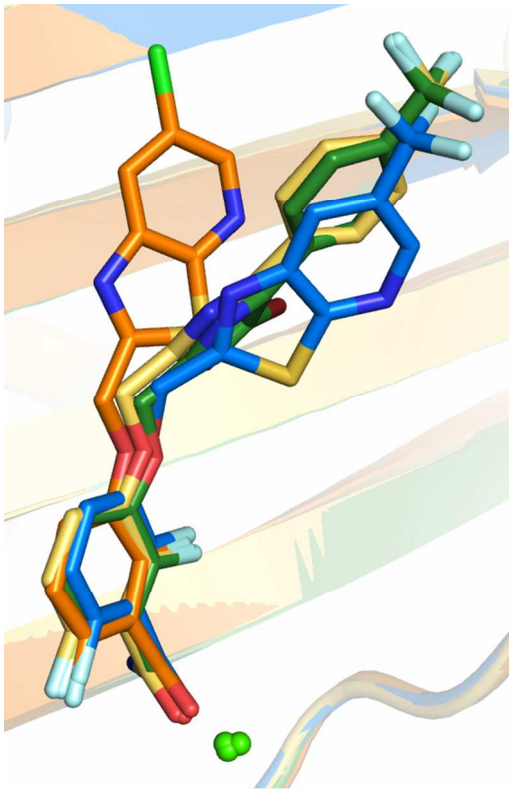


Figure S4. Superposition of the crystal structures of TXA707, TXA6101, and PC190723 in complex with SaFtsZ. TXA707 in complex with wildtype SaFtsZ is shown in blue, TXA6101 in complex with wildtype SaFtsZ is shown in yellow, TXA6101 in complex with G196S mutant SaFtsZ is shown in green, and PC190723 in complex with wildtype SaFtsZ is shown in orange (PDB entry: 4DXD).