Supporting Information

Structural Basis of the Promiscuous Inhibitor Susceptibility of *E. coli* LpxC

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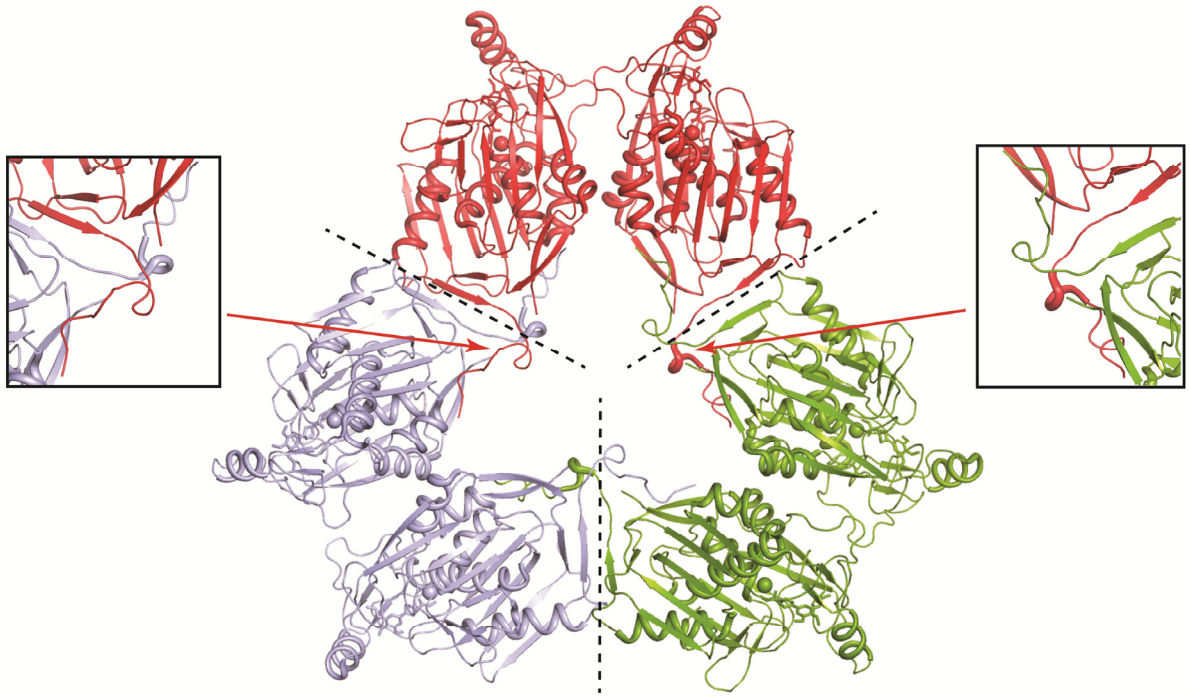

Figure S1. Crystallographic hexamer of the EcLpxC/L-161,240 complex. The C-terminal tails of the EcLpxC molecules are located in the water space facing the center of the crystallographic threefold rotation axis and make crystal contacts with symmetry-related neighboring molecules. Detailed views of the domain-swapped C-terminal tails around the crystal packing interfaces are shown in side panels. The three-fold symmetry related packing interfaces are shown as dashed lines, and the crystallographic pseudodimers of the EcLpxC/L-161,240 complex are colored in red, green and light purple, respectively.

Synthesis and characterization of LPC-138 and intermediates

LC/MS analysis was conducted on an Agilent 1200 HPLC with a quadrupole mass analyzer. LC chromatography used an Agilent XDB-C18 column (4.6×50 mm, 1.8 μm) with a water/acetonitrile (each with 0.2% (v/v) formic acid) gradient at a flow rate of 0.5 mL/min. HRMS analyses were performed at the Duke MS Center. ^{1}H and ^{13}C spectra were recorded at 300 (400) and 75 (100) MHz, respectively, on a Varian Spectrometer. Column chromatography was conducted using either silica gel (Silicycle 40-64 μm) or prepacked RediSep columns (Teledyne Isco Inc., Lincoln, NE) on an Isco CombiFlash Rf instrument. HPLC purification was performed on an Agilent 1200 system, equipped with a variable wavelength detector, using an Agilent Prep-C18 column (21.2x250 mm, 10 μm), eluted with a water/methanol (each 0.2% (v/v) formic acid) at a flow rate of 20 mL/min. All moisture-sensitive reactions were carried out using dry solvents and under a slight pressure of ultra-pure quality argon. Glassware was dried in an oven at 140°C for at least 12h prior to use, and then assembled quickly while hot, sealed with rubber septa, and allowed to cool under a stream of argon. Reactions were stirred magnetically using Teflon-coated magnetic stirring bars. Commercially available disposable syringes were used for transferring reagents and solvents.

(2R,3S,7R,7aS)-N-(benzyloxy)-7-methoxy-2,3,7,7a-tetramethyl-5-oxotetrahydro-2H-oxazolo[4,3-b]oxazole-3-carboxamide (2)

A stirred suspension of methyl (2R,3S,7R,7aS)-7-methoxy-2,3,7,7a-tetramethyl-5-oxotetrahydro-2H-oxazolo[4,3-b]oxazole-3-carboxylate[1] **(1)** (1.4 g, 5.1 mmol) and *O*-benzylhydroxylamine hydrochloride (899 mg, 5.6 mmol) in THF (20 mL) at -78 ^{o}C and under argon atmosphere was treated with a 1 M solution of LiHMDS in THF (18.4 mL, 18.4 mmol).

The mixture was stirred for 1h at -78 ^{o}C, after which the reaction was quenched with a saturated aqueous solution of NH_4Cl (10 mL). The contents were partitioned between EtOAc (25 mL) and water (20 mL). The aqueous layer was separated and extracted with EtOAc (15 mL). The organic layers were combined, washed with brine (20 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue obtained was purified by silica gel chromatography with ethyl acetate/hexanes (0 to 50% gradient) as eluant to afford (2R,3S,7R,7aS)-N-(benzyloxy)-7-methoxy-2,3,7,7a-tetramethyl-5-oxotetrahydro-2H-oxazolo[4,3-b]oxazole-3-carboxamide as an off-white solid (1.5 g, 81%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 9.26 (s, 1H), 7.42−7.34 (m, 5H), 4.93 (AB_q, J_{AB} = 10.8 Hz, 2H), 4.67 (q, J = 6.4 Hz, 1H), 3.45 (s, 3H), 1.53 (s, 3 H), 1.52 (s, 3H), 1.40 (d, J = 6.4 Hz, 3H), 1.32 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.1, 155.5, 134.8, 129.3, 128.9, 128.6, 108.8, 99.4, 80.6, 78.3, 66.3, 51.8, 18.1, 16.7, 16.6, 15.7.

(2S,3R)-2-amino-N-(benzyloxy)-3-hydroxy-2-methylbutanamide (3)

To a stirred solution of compound **2** (1.15 g, 3.16 mmol) in methanol (15 mL) was added 2M aqueous HCl (15 mL). The resulting mixture was stirred at RT for 8h and then heated at reflux temperature for additional 4h. The contents were cooled to RT and concentrated in vacuo to remove the methanol. The aqueous layer was extracted with Et_2O (2x15 mL). Solid Na_2CO_3 was then added to the aqueous layer with stirring to adjust the pH ~9 and extracted with EtOAc (3x20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo to afford (2S,3R)-2-amino-N-(benzyloxy)-3-hydroxy-2-methylbutanamide as a light brown liquid (410 mg, 55%). MS: 239 (M+H).

Perfluorophenyl 4-((4-aminophenyl)buta-1,3-diynyl)benzoate (5)

To a solution of 4-((4-aminophenyl)buta-1,3-diynyl)benzoic acid[2] (400 mg, 1.52 mmol) in anhydrous THF (25 mL) was added pentafluorophenol (784 mg, 4.56 mmol). The mixture was cooled to 0 ^{o}C and added with DCC (344 mg, 1.68 mmol) and DMAP (56 mg, 0.44 mmol) and stirred at the same temperature for 1 h, after which was allowed warm to room temperature gradually overnight (15h). The yellow suspension was filtered and washed with DCM (2x60 mL). The filtrate was concentrated to dryness in vacuo. The residue was added with water (60 mL) and extracted with EtOAc (2x60 mL). The combined organic layers were washed with water (2x75 mL) and brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue obtained was purified by silica gel chromatography with DCM as eluant to afford perfluorophenyl 4-((4-aminophenyl)buta-1,3-diynyl)benzoate as a yellow solid (Yield 625 mg; 89%). MS: 428 (M+H).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 6.62 (d, J = 8.0 Hz, 2H), 3.96 (s, 2H). ^{19}F NMR (375 MHz, $CDCl_3$): δ -152.37 (d, *J* = 18.0 Hz), -157.70 (t, *J* = 21.8 Hz), -162.16 (dd, *J* = 18.0 Hz, J = 21.8 Hz).

4-((4-aminophenyl)buta-1,3-diynyl)-N-((2S,3R)-1-(benzyloxyamino)-3-hydroxy-2-methyl-1-oxobutan-2-yl)benzamide (6)

To a stirred solution of the amino acid hydroxamate **3** (238 mg, 1.0 mmol) in anhydrous DMF (4 mL) under an atmosphere of argon, was added the perfluorophenyl ester **5** (385 mg, 0.9 mmol) followed by triethylamine (0.5 mL). The mixture was stirred at room temperature for 16 h. The contents were partitioned between EtOAc (25 mL) and water (20 mL). The aqueous layer was separated and extracted with EtOAc (15 mL). The organic layers were combined,

washed with H_2O (2x20 mL) and brine (20 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue obtained was purified by silica gel chromatography with ethyl acetate/hexanes (0 to 100% gradient) as eluant to afford 4-((4-aminophenyl)buta-1,3-diynyl)-N-((2S,3R)-1-(benzyloxyamino)-3-hydroxy-2-methyl-1-oxobutan-2-yl)benzamide as a foamy yellow solid (350 mg, 81%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 10.11 (s, 1H), 7.70 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 8.0 Hz, 2H), 7.44 – 7.30 (m, 7H), 6.61 (d, J = 8.0 Hz, 2H), 4.93 (s, 2H), 4.64 (m, 1H), 3.94 (br s, 2H), 3.57 (br s, 1H), 1.65 (s, 3H), 1.19 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.8, 167.0, 147.8, 135.0, 134.2, 133.8, 132.5, 129.3, 128.8, 128.6, 127.1, 126.2, 114.6, 110.2, 84.5, 79.6, 78.2, 77.3, 71.8, 68.9, 62.9, 20.9, 17.0.

4-((4-aminophenyl)buta-1,3-diynyl)-N-((2S,3R)-3-hydroxy-1-(hydroxyamino)-2-methyl-1-oxobutan-2-yl)benzamide (LPC-138)

A stirred solution of the benzyl protected hydroxamate **6** (241 mg, 0.5 mmol) in anhydrous DCM (10 mL) under an atmosphere of argon was cooled to -10 ^{o}C using ice-salt bath. Then 1M solution of BCl3 in DCM was added slowly and the resulting mixture was stirred for 1h while maintaining the temperature at -10 ^{o}C. The reaction was quenched with a saturated aqueous solution of NH_4Cl (10 mL). The contents were partitioned between EtOAc (30 mL) and water (10 mL). The aqueous layer was separated and extracted with EtOAc (15 mL). The organic layers were combined, washed with brine (20 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue obtained was purified by silica gel chromatography with methanol/DCM (0 to 10% gradient) as eluant to afford LPC-138 as yellow solid (110 mg, 56%).

^{1}H NMR (400 MHz, CD_3OD): δ 7.80 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 8.4 Hz, 2H), 6.62 (d, J = 8.4 Hz, 2H), 4.18 (q, *J* = 6.4 Hz, 1H), 1.58 (s, 3H), 1.19 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, CDCl3): δ 170.6, 167.0, 149.9, 134.2, 133.6, 131.9, 127.1, 125.7, 114.0, 108.1, 84.4, 78.8, 76.5, 70.6, 70.0, 63.4, 16.5, 16.6.

References for Supporting Information:

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