Supplementary Information

New Analyses of MIC_{90} Data to Aid Antibacterial Drug Discovery

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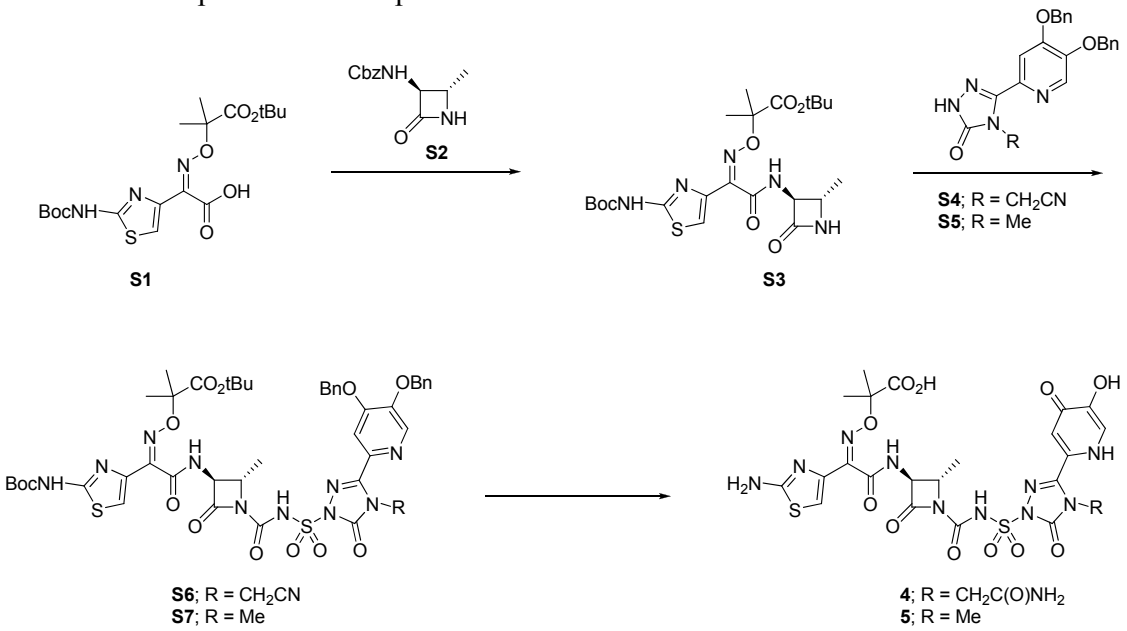
Compound Preparation and Characterization. Reagents and solvents were obtained from commercial sources unless otherwise noted. All reactions were run under nitrogen unless otherwise noted. Routine ^{1}H NMR spectra were recorded on a Varian Inova 400 MHz spectrometer unless otherwise specified. Low resolution mass spectral data was collected using a Waters Micromass ZMD (electrospray ionization, chromatography on a Varian Polaris 5 C-18 column with acetonitrile and 0.1% formic acid aqueous gradient eluant). The preparation of compounds **1** through **3** are described in the supporting information of Flanagan, M.E., Brickner, S.J. and Gootz, T.D., *et al., ACS Med. Chem. Lett.*, **2011**, *2*, 385-390. See also WO 2010070523 A1 for additional experimentals. Compounds **4** and **5** were prepared as outlined in Scheme S1.

Scheme S1. Preparation of compounds **4** and **5**.



tert-butyl-2-((Z)-1-(2-(tert-butoxycarbonylamino)thiazol-4-yl)-2-((2S,3S)-2-methyl-4-oxoazetidin-3-ylamino)-2-oxoethylideneaminooxy)-2-methylpropanoate (**S3**). To a suspension of (*Z*)-2-(1-*tert*-butoxy-2-methyl-1-oxopropan-2-yloxyimino)-2-(2-(tert-butoxycarbonylamino) thiazol-4-yl)acetic acid **S1** (102.46 g, 238 mmol, 1 eq) prepared by the method of Yamawaki, K., *et al. Bioorg. and Med. Chem Lett*., **2007**, *15*, 6716 and *N*-hydroxysuccinimide (30.2 g, 262 mmol, 1.1 eq) in DCM (1L) was dropwise added at 0 °C) a solution of diisopropylcarbodiimide (DIC) (39 mL), 1.05 eq) in DCM (40 mL). The mixture was stirred for 30 min at 0°C followed by 2h at room temperature. The mixture was filtered through Celite to remove solids. The filtrate was concentrated *in vacuo* to give the crude product which was stirred in isopropanol (550 mL) for 30 min, filtered and washed with cold diethyl ether (100 mL) to provide the *N*-hydroxysuccinamide ester (123.8 g, 235 mmol) as a white powder in 98% yield. Separately, to a solution of benzyl (2*S*,3*S*)-2-methyl-4-oxoazetidin-3-yl carbamate **S2** (40 g, 171 mmol) prepared by the method of Miller, M.J., *et al. J. Org. Chem.,* **1986**, *51*, 3133 in acetic acid (400 mL) was added Pd/C (10% unreduced, Acros) (1.4 g). The mixture was stirred (1500 rpm) in Parr

apparatus for 1h at 5 bar under H_2 atm. The mixture was filtered over Celite and washed with some additional acetic acid. The acetic acid was removed as fast as possible on a rotavap. A mixture containing of the amine as the acetate salt (55.56 g) was collected and moved forward without further purification. The crude mixture of the acetate salt (55.56 g, max 171 mmol) was dissolved in acetonitrile (350 mL), and to this mixture added the NHS-activated ester (88.7 g, 168 mmol, 0.98 eq) and the solution cooled to 0°C. Triethylamine (89 mL, 641 mmol, 3.7 eq) was added dropwise (T < 5°C). The mixture was stirred at room temperature for 16h. The reaction was quenched by the addition of water (350 mL) and stirred for 1h. The mixture was extracted with EtOAc (3x 250 mL). The combined organic phases were washed with brine, dried over sodium sulfate and concentrated *in vacuo* to yield a light brown foam (95.89 g) with a purity of 64% (LCMS). The mixture was purified by column chromatography (silica 2.5 kg, TEA treatment), eluent: DCM: THF (4:1) which yielded **S3** in two batches. Batch 1 (17.8 g, 34.8 mmol) with 92% purity (HPLC-MS) and batch 2 (37.1 g, 73 mmol) with 96% purity. To remove the traces THF, both batches were dissolved in EtOAc and heptane was added while stirring. EtOAc was removed *in vacuo* and mixture was cooled to RT. Product **S3** (B1: 17.11 g, 33.4 mmol, 92% pure; B2: 35.45 g, 69.3 mmol, 96% pure) was filtered off in 57% yield. LCMS *m/z*: 512.3 (MH+). ^{1}H NMR (400 MHz)(DMSO-*d6*) δ: 1.26 (3H, d, J = 6.1 Hz), 1.34 (18H, s), 1.41 (6H, s), 3.52 (1H, q, J = 2.1 Hz), 4.40 (1H, dd, J = 8.2 Hz, J = 6.2 Hz), 7.20 (1H, s), 8.15 (1H, s), 8.96 (1H, d, J = 8.0 Hz), 11.76 (1H, s).

tert-butyl-2-((Z)-2-((2S,3S)-1-(3-(4,5-bis(benzyloxy)pyridin-2-yl)-4-(cyanomethyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-ylsulfonylcarbamoyl)-2-methyl-4-oxoazetidin-3-ylamino)-1-(2-(tert-butoxycarbonylamino)thiazol-4-yl)-2-oxoethylideneaminooxy)-2-methylpropanoate (**S6**). A

mixture of **S4** (1.05 g, 2.54 mmol) prepared as described in WO 2010070523 A1 in tetrahydrofuran (8 mL) was treated with 2,2,2-trifluoro-*N*-methyl-*N*-(trimethylsilyl)acetamide (MSTFA, 98%, 2.9 mL, 15.2 mmol). After 45 minutes of stirring, the light yellow milky mixture was concentrated *in vacuo* at 60°C for 1 hour, then dried under vacuum at 60°C for 1.5 hours. In a separate flask, a suspension of **S3** (1.30 g, 2.54 mmol) in dichloromethane (8 mL) was cooled to 0°C, treated drop-wise with chlorosulfonyl isocyanate (95%, 0.265 mL, 3.05 mmol) and allowed to stir for 30 minutes under ice-cooling. The material derived from **S4** was dissolved in tetrahydrofuran (8 mL), cooled to 0°C. The ice-cooled reaction mixture of **S3** was then transferred into this solution via cannula. After stirring at 0°C for 1 hour, then at room temperature for 1.5 hours, the reaction mixture was quenched with methanol (5 mL), stirred for 10 minutes and concentrated *in vacuo*. The residue was purified by silica gel chromatography (Gradient: 40-100% ethyl acetate in heptane, then 0-12% methanol in ethyl acetate) to afford 2.57 g (29%) of **S6** as a solid. LCMS *m/z*: 1031.4 (MH+). ^{1}H NMR (400 MHz)(DMSO-*d6*) δ: 1.30 (9H, s), 1.44 (9H, s), 3.86 – 3.88 (1H, m), 4.42 – 4.62 (1H, m), 5.15 (2H, br. s), 5.25 (2H, s), 5.27 (2H, s), 7.22 (1H, s), 7.30 – 7.45 (10H, m), 7.58 (1H, s), 8.40 (1H, s), 9.00 (1H, d, *J* = 7.6 Hz).

tert-butyl-2-((Z)-2-((2S,3S)-1-(3-(4,5-bis(benzyloxy)pyridin-2-yl)-4-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-ylsulfonylcarbamoyl)-2-methyl-4-oxoazetidin-3-ylamino)-1-(2-(tert-butoxycarbonylamino)thiazol-4-yl)-2-oxoethylideneaminooxy)-2-methylpropanoate (**S7**). This intermediate was prepared in an analogous manner to that described for **S6**, but replacing **S4** with 3-(4,5-bis(benzyloxy)pyridin-2-yl)-4-methyl-1H-1,2,4-triazol-5(4H)-one (**S5**) prepared by the method of Barbachyn, M.R., *et al., J. Anitibiotics,* **1990**, *43*(9), 1199 resulting in 360 mg (22%)

as a light pink solid. LCMS *m/z*: 1005.9 (MH+). ^{1}H NMR (400 MHz)(DMSO-*d6*) δ: 1.35 (9H, s), 1.43 (9H, s), 3.14 (3H, s), 3.78 – 3.80 (1H, m), 4.43 (1H, dd, *J* = 10.7 Hz, *J* = 2.3 Hz), 5.27 (2H, s), 5.28 (2H, s), 7.22 (1H, s), 7.30 – 7.44 (10H, m), 7.57 (1H, s), 8.38 (1H, s), 8.99 (1H, d, *J* = 7.8 Hz), 11.79 (1H, br. s).

2-((Z)-2-((2S,3S)-1-(4-(2-amino-2-oxoethyl)-3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-ylsulfonylcarbamoyl)-2-methyl-4-oxoazetidin-3-ylamino)-1-(2-aminothiazol-4-yl)-2-oxoethylideneaminooxy)-2-methylpropanoic acid (**4**). A solution of **S6** (1.03 g, 0.725 mmol) in tetrahydrofuran (25 mL) and acetic acid (0.25 mL) was degassed and flushed with nitrogen (3x) and treated with Pd black (134 mg). The mixture was hydrogenated using a Parr shaker under 36 psi hydrogen at room temperature for 4 hours (reaction complete by LCMS). The sample was filtered through acid washed cellulose powder and washed with THF to give a pale red filtrate, which was concentrated to dryness in vacuo affording 0.646 g of the debenzylated intermediate as a red solid. Trifluoroacetic acid (3.5 mL) was added to a cooled (0^{o}C) solution of the debenzylated intermediate in 3.5 mL of dichloromethane. The reaction mixture was stirred at room temperature for 2 hours and then transfered slowly via a teflon cannula to another round bottom flask containing 186 mL of a 2:1 mixture of heptane/methyl-t-butyl ether (MTBE) resulting in a fine precipitate. The solids were collected, washed with heptane/MTBE (2:1) and dried in vacuo affording the crude trifluoroacetic acid salt of **4** as a rose colored solid. This material was then purified by reverse phase chromatography using an Isco Rf Chromatography system employing a RediSep Rf C18 column (130 g), loading the crude trifluoroacetic acid salt as a solution in dimethylsulfoxide (1.5 mL) in two batches. The gradient was 5% to 30% water (0.1% Formic acid)/acetonitrile (0.1% Formic acid). The product came off the column at 15-18% acetonitrile. The fractions were

pooled and the solvent was removed under reduced pressure. Sonication in methanol was used to drive off remaining formic acid affording 0.104 g (19%) of material as a white solid. LCMS *m/z:* 712.6 (M+1). ^{1}H NMR (400 MHz) (DMSO-*d6*) δ: 1.39 (3H, s), 1.41 (6H, s), 2.47 – 2.51 (2H, m), 3.76 (1H, d, *J* = 4.0 Hz), 4.44 (1H, dd, *J* = 8.0 Hz, *J* = 3.1 Hz), 4.60 (1H, s), 6.81 (1H, br. s), 7.04 (1H, br. s), 7.32 (1H, s), 7.34 (1H, br. s), 7.91 (1H, s), 9.05 (1H, d, *J* = 8.0 Hz).

2-((Z)-1-(2-aminothiazol-4-yl)-2-((2S,3S)-1-(3-(5-hydroxy-4-oxo-1,4-dihydropyridin-2-yl)-4-methyl-5-oxo-4,5-dihydro-1H-1,2,4-triazol-1-ylsulfonylcarbamoyl)-2-methyl-4-oxoazetidin-3-ylamino)-2-oxoethylideneaminooxy)-2-methylpropanoic acid (**5**). Conversion of **S7** to **5** was carried out in an analogous manner to that described for the conversion of **S6** to **4** affording 84 mg (33%) of **5** as a lite pink solid. LCMS *m/z:* 669.7 (M+1). ^{1}H NMR (400 MHz) (DMSO-*d6*) δ: 1.40 (3H, s), 1.41 (3H, s), 1.42 (3H, s), 3.36 (3H, s), 3.77 (1H, dd, *J* = 6.2 Hz, *J* = 3.3 Hz), 4.44 (1H, dd, *J* = 8.2 Hz, *J* = 5.5 Hz), 6.81 1H, s), 7.34 (1H, s), 8.00 (1H, s), 9.06 (1H, d, *J* = 8.0 Hz).