

Identification of inhibitors of PvdQ, an enzyme involved in the synthesis of the siderophore pyoverdine

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Chemistry. General protocols.

All reagents and solvents were purchased from commercial vendors and used as received. NMR spectra were recorded at 21 °C on a Bruker 300 MHz instrument. Proton and carbon chemical shifts are reported in parts per million (δ) relative to trimethylsilane (^1H ; $\delta 0$) and CDCl_3 (^{13}C ; $\delta 77.0$), respectively. NMR data are reported as follows: chemical shift (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constant(s) in Hz; integration). Flash chromatography was performed using 40–60 μm Silica Gel (60 Å mesh) on a Teledyne Isco Combiflash R_f system. Tandem liquid chromatography-mass spectrometry (LCMS) was performed on a Waters 2795 separations module and Waters 3100 mass detector. For high-resolution mass measurements, liquid chromatography/mass spectrometry (LCMS) was performed on an Agilent 1290 Infinity separations module and 6230 time-of-flight (TOF) mass detector operating in ESI+ mode. Compound purity and identity were determined by UPLC-MS and LCMS. Purity was measured by UV absorbance at 210 nm (UPLC) and 254 nm (LCMS). For UPLC-MS: Identity was determined on a SQ mass spectrometer by electrospray ionization. Mobile Phase A consisted of either 0.1% ammonium hydroxide or 0.1% trifluoroacetic acid in water, while mobile Phase B consisted of the same additives in acetonitrile. The gradient ran from 5% to 95% mobile Phase B over 0.8 minutes at 0.45 mL/min. An Acquity BEH C18, 1.7 μm , 1.0 x 50 mm column was used with column temperature maintained at 65°C. Compounds were dissolved in DMSO at a nominal concentration of 1 mg/mL, and 0.25 μL of this solution was injected.

General Protocol A.

A solution of phenylacetonitrile (1 equiv) and 2-bromo- or 2-chloropyridine (1.1 equiv) in dry DMF (0.15 M) was cooled to 0 °C. Potassium *tert*-butoxide (2.0 equiv) as a solution in DMF

(0.8 M) was added dropwise. The reaction mixture was stirred for 3 h at 0 °C and then warmed to rt over 30 min. The reaction was quenched with satd aq NH₄Cl, extracted with ether, washed with water, dried over MgSO₄, filtered and concentrated onto silica gel. The crude product was purified by flash chromatography using ethyl acetate in hexanes.

2-(4-Fluorophenyl)-2-(6-(trifluoromethyl)pyridin-2-yl) acetonitrile (4). The title compound was prepared using General Procedure A (43 mg, 69%). ¹H NMR (300 MHz, CDCl₃) δ 7.93 (t, *J* = 7.9 Hz, 1H), 7.64 (dd, *J* = 12.2, 7.9 Hz, 2H), 7.47 (dd, *J* = 8.6, 5.1 Hz, 2H), 7.09 (t, *J* = 8.6 Hz, 2H), 5.40 (s, 1H). ¹³C NMR (75 MHz, CDCl₃) δ 164.57, 161.27, 156.22, 148.59 (q, *J* = 35.2 Hz), 139.42, 129.79 (d, *J* = 3.4 Hz), 129.58 (d, *J* = 8.4 Hz), 121.16 (q, *J* = 273.0 Hz), 124.66, 120.13 (q, *J* = 2.7 Hz), 118.33, 116.67, 116.38, 77.36, 44.54. HRES MS *m/z*: (pos) 280.0628. UPLC purity: 91% *m/z*: (pos) 281.17; (neg) 279.20.

2-(6-Chloropyridin-2-yl)-2-(4-fluorophenyl)aceto-nitrile (9). The title compound was prepared using General Procedure A (17 mg, 20%). ¹H NMR (300 MHz, CDCl₃) δ 7.90 (t, *J* = 7.9 Hz, 1H), 7.67 (dd, *J* = 7.2, 6.0 Hz, 2H), 7.45–7.35 (m, 3H), 5.34 (s, 1H). UPLC purity: 91% *m/z*: (pos) 247.37; (neg) 245.28.

2-(6-Chloropyridin-2-yl)-2-(4-(trifluoromethyl) phenyl)acetonitrile (10). The title compound was prepared using General Procedure A (38 mg, 37%). ¹H NMR (300 MHz, CDCl₃) δ 7.67 (dt, *J* = 17.5, 8.0 Hz, 1H), 7.39 (d, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 5.34 (s, 1H). LCMS purity: 90% *m/z*: (pos) 297.16; (neg) 295.19.

2-(6-Chloropyridin-2-yl)-2-(2-(trifluoromethoxy)-phenyl)acetonitrile (11). The title compound was prepared using General Procedure A (46 mg, 46%). ¹H NMR (300 MHz, CDCl₃)

δ 7.72–7.64 (m, 1H), 7.40 (dq, $J = 14.9, 7.5, 1.5$ Hz, 1H), 7.30 (d, $J = 8.4$ Hz, 1H), 5.56 (s, 1H).

UPLC purity: 96% m/z : (pos) 313.12; (neg) 311.16.

2-(6-Chloropyridin-2-yl)-2-(2,4-dichlorophenyl) acetonitrile (12). The title compound was prepared using General Procedure A (28 mg, 18%). ^1H NMR (300 MHz, CDCl_3) δ 7.68 (t, $J = 7.8$ Hz, 1H), 7.58 (d, $J = 8.4$ Hz, 1H), 7.45 (d, $J = 2.1$ Hz, 1H), 7.32 (ddd, $J = 27.4, 13.9, 4.9$ Hz, 3H), 5.66 (s, 1H). UPLC purity: 91% m/z : (pos) 297.07; (neg) 295.10.

2-(4-Chloro-2-fluorophenyl)-2-(6-chloropyridin-2-yl) acetonitrile (13). The title compound was prepared using General Procedure A (62 mg, 63%). ^1H NMR (300 MHz, CDCl_3) δ 7.70 (t, $J = 7.8$ Hz, 1H), 7.50 (t, $J = 8.2$ Hz, 1H), 7.33 (dd, $J = 9.5, 8.0$ Hz, 2H), 7.23 (dd, $J = 8.0, 1.6$ Hz, 1H), 7.15 (dd, $J = 9.7, 2.0$ Hz, 1H), 5.47 (s, 1H). UPLC purity: 100% m/z : (pos) 281.19.

2-(4-Chlorophenyl)-2-(5-chloropyridin-2-yl) acetonitrile (14). The title compound was prepared using General Procedure A (71 mg, 50%). ^1H NMR (300 MHz, CDCl_3) δ 8.54 (d, $J = 2.4$ Hz, 1H), 7.69 (dd, $J = 8.4, 2.5$ Hz, 1H), 7.36 (s, 4H), 5.28 (s, 1H). UPLC purity: 92% m/z : (pos) 263.09; (neg) 261.15.

2-(4-Chlorophenyl)-2-(3-chloropyridin-2-yl) acetonitrile (15). The title compound was prepared using General Procedure A (45 mg, 32%). ^1H NMR (300 MHz, CDCl_3) δ 7.72 (dd, $J = 8.1, 1.5$ Hz, 1H), 7.39 (dd, $J = 30.9, 8.5$ Hz, 4H), 7.27 (dd, $J = 8.2, 4.6$ Hz, 1H), 5.75 (s, 1H). UPLC purity: 100% m/z : (pos) 263.11; (neg) 261.15.

2-(4-Chlorophenyl)-2-(6-(trifluoromethyl)pyridin-2-yl)acetonitrile (16). The title compound was prepared using General Procedure A (74 mg, 83%). ESI MS m/z : (pos) 297.0; (neg) 294.9. ^1H NMR (300 MHz, CDCl_3) δ 7.93 (t, $J = 7.9$ Hz, 1H), 7.64 (dd, $J = 13.1, 7.9$ Hz, 2H), 7.47–7.29 (m, 4H), 5.39 (s, 1H). LCMS purity: 98% m/z : (pos) 297.11; (neg) 295.15.

6-((4-Chlorophenyl)(cyano)methyl)picolinonitrile (17). The title compound was prepared using General Procedure A (49 mg, 36%). ¹H NMR (300 MHz, CDCl₃) δ 7.90 (t, *J* = 7.9 Hz, 1H), 7.67 (dd, *J* = 7.2, 6.0 Hz, 2H), 7.45–7.35 (m, 3H), 5.34 (s, 1H). UPLC purity: 80% *m/z*: (pos) 254.16; (neg) 252.19.

2-(4-Chlorophenyl)-2-(6-methylpyridin-2-yl)aceto-nitrile (18). The title compound was prepared using General Procedure A (54 mg, 4%). ¹H NMR (300 MHz, CDCl₃) δ 7.58 (t, *J* = 7.7 Hz, 1H), 7.42–7.30 (m, 4H), 7.16 (d, *J* = 7.7 Hz, 1H), 7.13–7.07 (m, 1H), 5.25 (s, 1H), 2.55 (s, 3H). UPLC purity: 94% *m/z*: (pos) 243.15; (neg) 241.23.

2-(4-Chlorophenyl)-2-(5-methylpyridin-2-yl) aceto-nitrile (19). The title compound was prepared using General Procedure A (21 mg, 16%). ¹H NMR (300 MHz, CDCl₃) δ 8.42 (d, *J* = 0.7 Hz, 1H), 7.52 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.42–7.31 (m, 4H), 7.28 (d, *J* = 7.2 Hz, 1H), 5.25 (s, 1H), 2.33 (s, 3H). UPLC purity: 97% *m/z*: (pos) 243.16; (neg) 241.19.

2-(4-Chlorophenyl)-2-(5-(trifluoromethyl)pyridin-2-yl)acetonitrile (20). The title compound was prepared using General Procedure A (46 mg, 28%). ¹H NMR (300 MHz, CDCl₃) δ 8.86 (dd, *J* = 1.3, 0.8 Hz, 1H), 7.97 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.44–7.34 (m, 4H), 5.37 (s, 1H). UPLC purity: 94% *m/z*: (pos) 297.12.

2-(6-Chloro-5-(trifluoromethyl)pyridin-2-yl)-2-(4-chlorophenyl)acetonitrile (21). The title compound was prepared using General Procedure A (47 mg, 22%). ¹H NMR (300 MHz, CDCl₃) δ 8.05 (d, *J* = 8.0 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.45–7.35 (m, 4H), 5.32 (s, 1H). LCMS purity: 99% *m/z*: (pos) 331.05; (neg) 328.97.

6-((4-Chlorophenyl)(cyano)methyl)-3-methyl picolinonitrile (22). The title compound was prepared using General Procedure A (43 mg, 30%). ¹H NMR (300 MHz, CDCl₃) δ 7.71 (d, *J* =

8.3 Hz, 1H), 7.53 (d, $J = 8.2$ Hz, 1H), 7.40 (d, $J = 10.9$ Hz, 3H), 5.30 (s, 1H), 2.56 (s, 3H). UPLC purity: 88% m/z : (pos) 268.16; (neg) 266.20.

6-((4-Chlorophenyl)(cyano)methyl)nicotinonitrile (23). The title compound was prepared using General Procedure A (79 mg, 59%). ^1H NMR (300 MHz, CDCl_3) δ 8.85 (dd, $J = 2.0, 0.7$ Hz, 1H), 8.00 (dd, $J = 8.2, 2.1$ Hz, 1H), 7.54 (dd, $J = 8.2, 0.5$ Hz, 1H), 7.37 (s, 4H), 5.35 (s, 1H). UPLC purity: 97% m/z : (neg) 252.20.

2-(4-Chlorophenyl)-2-(pyridin-2-yl)acetonitrile (24). The title compound was prepared using General Procedure A (407 mg, 23%). ^1H NMR (300 MHz, CDCl_3) δ 8.67–8.51 (m, 1H), 7.72 (td, $J = 7.8, 1.7$ Hz, 1H), 7.47–7.30 (m, 5H), 7.26 (t, $J = 6.2$ Hz, 1H), 5.29 (s, 1H). UPLC purity: 92% m/z : (pos) 229.39; (neg) 227.26.

6-(Cyano(4-fluorophenyl)methyl)picolinonitrile (25). The title compound was prepared using General Procedure A (19 mg, 23%). ^1H NMR (300 MHz, CDCl_3) δ 7.90 (t, $J = 7.9$ Hz, 1H), 7.67 (dd, $J = 7.5, 5.2$ Hz, 2H), 7.45 (dd, $J = 8.6, 5.0$ Hz, 2H), 7.10 (t, $J = 8.6$ Hz, 2H), 5.35 (s, 1H). UPLC purity: 83% m/z : (pos) 238.40; (neg) 236.32.

6-(Cyano(4-fluorophenyl)methyl)-3-methylpicolino-nitrile (26). The title compound was prepared using General Procedure A (29 mg, 33%). ^1H NMR (300 MHz, CDCl_3) δ 7.71 (d, $J = 8.2$ Hz, 1H), 7.53 (d, $J = 8.2$ Hz, 1H), 7.43 (dd, $J = 8.6, 5.0$ Hz, 2H), 7.09 (t, $J = 8.6$ Hz, 2H), 5.31 (s, 1H). UPLC purity: 82% m/z : (pos) 252.14; (neg) 250.24.

2-(6-Chloro-5-(trifluoromethyl)pyridin-2-yl)-2-(4-fluorophenyl)acetonitrile (27). The title compound was prepared using General Procedure A (74 mg, 67%). ^1H NMR (300 MHz, CDCl_3) δ 8.05 (d, $J = 8.0$ Hz, 1H), 7.58–7.41 (m, 3H), 7.11 (t, $J = 8.6$ Hz, 2H), 5.32 (s, 1H). LCMS purity: 97% m/z : (pos) 315.14; (neg) 313.13.

2-(6-Chloro-5-(trifluoromethyl)pyridin-2-yl)-2-(4-(trifluoromethyl)phenyl)acetonitrile (28).

The title compound was prepared using General Procedure A (56 mg, 44%). ¹H NMR (300 MHz, CDCl₃) δ 8.07 (d, *J* = 8.0 Hz, 1H), 7.66 (dd, *J* = 20.3, 8.4 Hz, 4H), 7.54 (d, *J* = 7.9 Hz, 1H), 5.40 (s, 1H). LCMS purity: 100% *m/z*: (pos) 365.12; (neg) 363.12.

2-(4-(Trifluoromethyl)phenyl)-2-(6-(trifluoromethyl)pyridin-2-yl)acetonitrile (29).

The title compound was prepared using General Procedure A (50 mg, 43%). ¹H NMR (300 MHz, CDCl₃) δ 7.95 (t, *J* = 7.9 Hz, 1H), 7.72–7.59 (m, 6H), 5.48 (s, 1H). UPLC purity: 97% *m/z*: (pos) 331.13.

6-((4-Chloro-2-fluorophenyl)(cyano)methyl)picolinonitrile (30).

The title compound was prepared using General Procedure A (50 mg, 53%). ¹H NMR (300 MHz, CDCl₃) δ 7.92 (t, *J* = 7.9 Hz, 1H), 7.72 – 7.63 (m, 2H), 7.54 (t, *J* = 8.2 Hz, 1H), 7.24 (d, *J* = 1.2 Hz, 2H), 7.16 (dd, *J* = 9.8, 2.0 Hz, 1H), 5.54 (s, 1H). LCMS purity: 100% *m/z*: (pos) 272.15; (neg) 270.15.

2-(4-Chloro-2-fluorophenyl)-2-(5-(trifluoromethyl)pyridin-2-yl)acetonitrile (31).

The title compound was prepared using General Procedure A (62 mg, 56%). ¹H NMR (300 MHz, CDCl₃) δ 7.94 (t, *J* = 7.9 Hz, 1H), 7.64 (dd, *J* = 15.3, 7.8 Hz, 2H), 7.54 (t, *J* = 8.2 Hz, 1H), 7.26–7.22 (m, 1H), 7.16 (dd, *J* = 9.7, 2.0 Hz, 1H), 5.58 (s, 1H). UPLC purity: 91% *m/z*: (pos) 315.16.

2-(4-Chloro-2-fluorophenyl)-2-(6-chloro-5-(trifluoromethyl)pyridin-2-yl)acetonitrile (32).

The title compound was prepared using General Procedure A (70 mg, 57%). ¹H NMR (300 MHz, CDCl₃) δ 8.06 (d, *J* = 8.0 Hz, 1H), 7.61–7.48 (m, 2H), 7.25 (d, *J* = 1.2 Hz, 1H), 7.17 (dd, *J* = 9.7, 2.0 Hz, 1H), 5.52 (s, 1H). UPLC purity: 97% *m/z*: (pos) 288.33.

6-(Cyano(2,4-dichlorophenyl)methyl)picolinonitrile (33).

The title compound was prepared using General Procedure A (34 mg, 22%). ¹H NMR (300 MHz, CDCl₃) δ 7.90 (t, *J* = 7.9 Hz, 1H), 7.69 (d, *J* = 7.0 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.46 (d, *J* = 2.1

Hz, 1H), 7.39 (dd, $J = 8.4, 2.1$ Hz, 1H), 5.74 (s, 3H). UPLC purity: 84% m/z : (pos) 288.09; (neg) 286.13.

6-(Cyano(2,4-dichlorophenyl)methyl)nicotinonitrile (34). The title compound was prepared using General Procedure A (87 mg, 57%). ^1H NMR (300 MHz, CDCl_3) δ 8.88 (d, $J = 1.4$ Hz, 1H), 8.01 (dd, $J = 8.1, 2.1$ Hz, 1H), 7.60 (d, $J = 8.4$ Hz, 1H), 7.51 (d, $J = 8.4$ Hz, 1H), 7.47 (d, $J = 2.1$ Hz, 1H), 7.38 (dd, $J = 8.4, 2.1$ Hz, 1H), 5.77 (s, 1H). UPLC purity: 91% m/z : (neg) 286.11.

2-(3-Chloropyridin-2-yl)-2-(2,4-dichlorophenyl) acetonitrile (35). The title compound was prepared using General Procedure A (23 mg, 15%). ^1H NMR (300 MHz, CDCl_3) δ 8.54 (dd, $J = 4.7, 1.5$ Hz, 1H), 7.76 (dd, $J = 8.1, 1.5$ Hz, 1H), 7.52 (d, $J = 8.4$ Hz, 1H), 7.44 (d, $J = 2.1$ Hz, 1H), 7.36–7.27 (m, 2H), 6.10 (s, 2H). UPLC purity: 100% m/z : (pos) 297.06.

2-(6-Chloro-5-(trifluoromethyl)pyridin-2-yl)-2-(2,4-dichlorophenyl)acetonitrile (36). The title compound was prepared using General Procedure A (83 mg, 42%). ^1H NMR (300 MHz, CDCl_3) δ 8.05 (d, $J = 8.0$ Hz, 1H), 7.62 (d, $J = 8.4$ Hz, 1H), 7.41 (ddd, $J = 13.0, 8.7, 2.1$ Hz, 3H), 5.74 (s, 1H). UPLC purity: 89% m/z : (pos) 365.12; (neg) 363.11.

General Procedure B

Phenyl acetonitrile (1 equiv) was dissolved in dry DMF (0.15 M) and cooled to 0 °C. Sodium hydride (1.1 equiv) as a solution in DMF (0.8 M) was added dropwise. The mixture was stirred for 20 min and then alkyl chloride or bromide (5 equiv) was added. The reaction stirred for 1.5 h at 0 °C before warming to rt over 30 min. The reaction was quenched with satd aq NH_4Cl , extracted with ether, washed with water, dried over MgSO_4 , filtered and concentrated onto silica gel. The crude product was purified by flash chromatography using ethyl acetate in hexanes.

2-(4-Chlorophenyl)pentanenitrile (37). The title compound was prepared using General Procedure B (27 mg, 21%). ¹H NMR (300 MHz, CDCl₃) δ 7.31 (dd, *J* = 26.8, 8.5 Hz, 4H), 3.83–3.71 (m, 1H), 1.98–1.72 (m, 2H), 1.59–1.39 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H). UPLC purity: 100% *m/z*: (neg) 192.30.

2-(4-Chlorophenyl)hexanenitrile (38). The title compound was prepared using General Procedure B (24 mg, 17%). ¹H NMR (300 MHz, CDCl₃) δ 7.31 (dd, *J* = 27.7, 8.5 Hz, 4H), 3.80–3.71 (m, 1H), 1.95–1.76 (m, 2H), 1.51–1.28 (m, 4H), 0.91 (t, *J* = 7.1 Hz, 3H). UPLC purity: 100% *m/z*: (pos) 208.21.

2-(4-Chlorophenyl)-4-methylpentanenitrile (39). The title compound was prepared using General Procedure B (63 mg, 46%). ¹H NMR (300 MHz, CDCl₃) δ 7.31 (dd, *J* = 25.7, 8.5 Hz, 4H), 3.79 (dd, *J* = 9.5, 6.4 Hz, 1H), 1.95–1.70 (m, 2H), 1.61 (dd, *J* = 12.9, 6.5 Hz, 1H), 0.98 (dd, *J* = 6.4, 3.4 Hz, 6H). UPLC purity: 95% *m/z*: (pos) 208.19.

2-(4-Chlorophenyl)-3-cyclopropylpropanenitrile (40). The title compound was prepared using General Procedure B (8 mg, 6%). ¹H NMR (300 MHz, CDCl₃) δ 7.32 (dd, *J* = 22.7, 9.7 Hz, 4H), 3.88–3.81 (m, 1H), 1.93–1.79 (m, 1H), 1.76–1.65 (m, 1H), 0.89–0.72 (m, 1H), 0.54 (d, *J* = 7.6 Hz, 2H), 0.20–0.08 (m, 2H). UPLC purity: 100% *m/z*: (pos) 206.16; (neg) 204.21.

2-(4-Chlorophenyl)-4-methoxybutanenitrile (41). The title compound was prepared using General Procedure B (84 mg, 61%). ¹H NMR (300 MHz, CDCl₃) δ 7.32 (dd, *J* = 23.0, 8.6 Hz, 4H), 4.09–4.00 (m, 1H), 3.58–3.47 (m, 1H), 3.42–3.27 (m, 4H), 2.23–1.96 (m, 2H). UPLC purity: 100% *m/z*: (pos) 210.21; (neg) 208.20.

2-(4-Chlorophenyl)-4-(2-methoxyethoxy)butanenitrile (42). The title compound was prepared using General Procedure B (151 mg, 90%). ¹H NMR (300 MHz, CDCl₃) δ 7.33 (dd, *J* = 18.2,

8.5 Hz, 4H), 4.20–3.99 (m, 1H), 3.61 (dd, $J = 15.0, 9.2$ Hz, 5H), 3.52–3.29 (m, 4H), 2.30–1.96 (m, 2H). UPLC purity: 100% m/z : (pos) 255.41; (neg) 254.41.

2-(4-Chlorophenyl)-4-(dimethylamino)butanenitrile (43). The title compound was prepared using General Procedure B (78 mg, 50%). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.31 (dd, $J = 22.6, 8.4$ Hz, 4H), 3.84 (t, $J = 7.3$ Hz, 1H), 2.29 (t, $J = 6.9$ Hz, 2H), 2.17 (d, $J = 13.3$ Hz, 6H), 1.93 (dd, $J = 13.6, 7.2$ Hz, 2H), 1.62 (dd, $J = 14.2, 7.0$ Hz, 2H). UPLC purity: 94% m/z : (pos) 237.44.

2-(4-Fluorophenyl)hexanenitrile (46). The title compound was prepared using General Procedure B (65 mg, 55%). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.41–7.20 (m, 2H), 7.16–6.96 (m, 2H), 3.76 (dd, $J = 8.3, 6.5$ Hz, 1H), 2.05–1.72 (m, 2H), 1.56–1.19 (m, 4H), 0.91 (t, $J = 7.1$ Hz, 3H). UPLC purity: 99% m/z : (neg) 190.29.

2-(4-Fluorophenyl)-4-methoxybutanenitrile (47). The title compound was prepared using General Procedure B (54 mg, 38%). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.31 (dt, $J = 13.9, 7.0$ Hz, 2H), 7.08 (t, $J = 8.6$ Hz, 2H), 4.17–3.94 (m, 1H), 3.53 (dd, $J = 12.8, 9.2$ Hz, 1H), 3.46–3.20 (m, 4H), 2.28–1.93 (m, 2H). UPLC purity: 93% m/z : (neg) 192.28.

2-(4-(Trifluoromethyl)phenyl)hexanenitrile (48). The title compound was prepared using General Procedure B (102 mg, 68%). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.65 (d, $J = 8.2$ Hz, 2H), 7.47 (d, $J = 8.2$ Hz, 2H), 3.96–3.67 (m, 1H), 2.04–1.73 (m, 2H), 1.41 (tdd, $J = 35.2, 20.1, 14.9$ Hz, 4H), 0.91 (t, $J = 7.1$ Hz, 3H). UPLC purity: 100% m/z : (neg) 240.33.

2-(5-Chloropyridin-2-yl)hexanenitrile (49). The title compound was prepared using General Procedure B (94 mg, 69%). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 8.55 (d, $J = 2.4$ Hz, 1H), 7.72 (dd, $J = 8.3, 2.4$ Hz, 1H), 7.41 (d, $J = 8.3$ Hz, 1H), 3.98 (t, $J = 7.3$ Hz, 1H), 2.11–1.89 (m, 2H), 1.54–1.29 (m, 4H), 0.91 (t, $J = 7.1$ Hz, 3H). UPLC purity: 100% m/z : (pos) 209.42; (neg) 207.37.

2-(5-Fluoropyridin-2-yl)hexanenitrile (50). The title compound was prepared using General Procedure B (99 mg, 70%). ¹H NMR (300 MHz, CDCl₃) δ 8.45 (s, 1H), 7.52–7.37 (m, 2H), 3.99 (t, *J* = 7.3 Hz, 1H), 2.00 (dd, *J* = 15.2, 7.5 Hz, 2H), 1.43 (ddd, *J* = 19.3, 13.7, 6.9 Hz, 4H), 0.91 (t, *J* = 7.1 Hz, 3H). UPLC purity: 100% *m/z*: (pos) 193.46; (neg) 191.38.

2-(Pyridin-2-yl)hexanenitrile (51). The title compound was prepared using General Procedure B (106 mg, 72%). ¹H NMR (300 MHz, CDCl₃) δ 8.60 (d, *J* = 4.8 Hz, 1H), 7.74 (dd, *J* = 7.7, 6.1 Hz, 1H), 7.44 (d, *J* = 7.8 Hz, 1H), 7.26 (dd, *J* = 7.1, 5.1 Hz, 1H), 3.99 (t, *J* = 7.3 Hz, 1H), 2.02 (dd, *J* = 15.1, 7.6 Hz, 2H), 1.66–1.27 (m, 4H), 0.91 (t, *J* = 7.2 Hz, 3H). UPLC purity: 100% *m/z*: (pos) 175.45.

Procedures for analogs 44, 45, 52-55.

2-(4-Chlorophenyl)-2-((2-methoxyethyl)amino) aceto-nitrile (44). To a solution of sodium sulfite (210 mg, 1.6 mmol) and 1 N HCl (1.5 mL) in water (8.5 mL) was added 4-chlorobenzaldehyde (211 mg, 1.5 mmol). The reaction mixture was stirred for 15 minutes and 2-methoxyethanamine (0.14 mL, 1.6 mmol) was added. The reaction mixture was cooled over 45 min to 0 °C and 1 M aq sodium cyanide (3 mL) was added dropwise. The reaction mixture was stirred for 14 h gradually warming to room temperature. The reaction mixture was extracted with Et₂O (4 mL x 3), dried (Na₂SO₄), filtered and concentrated. The crude product was purified by silica flash chromatography with ethyl acetate and hexanes to deliver **44** (153 mg, 45%). ¹H NMR (300 MHz, CDCl₃) δ 7.48 (d, *J* = 8.5 Hz, 2H), 7.38 (d, *J* = 8.6 Hz, 2H), 4.85 (s, 1H), 3.55 (t, *J* = 5.0 Hz, 2H), 3.36 (s, 3H), 3.02–2.83 (m, 2H), 1.97 (s, 1H). UPLC purity: 85% *m/z*: (neg) 225.47.

2-(4-Chlorophenyl)-2-(pyrrolidin-1-yl)acetonitrile (45). To a solution of sodium sulfite (420 mg, 3.3 mmol) and 1 N HCl (3 mL) in water (17 mL) was added 4-chlorobenzaldehyde (422 mg, 3.0 mmol). The reaction mixture was stirred for 15 minutes and pyrrolidine (0.25 mL, 3.0 mmol) was added. The reaction mixture was cooled over 45 min to 0 °C and 1 M aq sodium cyanide (3 mL) was added dropwise. The reaction mixture was stirred for 14 h gradually warming to room temperature. The reaction mixture was extracted with Et₂O (3 x 4 mL), dried (Na₂SO₄), filtered and concentrated. The crude product was purified by silica flash chromatography with ethyl acetate and hexanes to deliver **45** (42 mg, 6%). ¹H NMR (300 MHz, CDCl₃) δ 7.42 (dd, *J* = 30.6, 8.5 Hz, 1H), 5.03 (s, 1H), 2.64 (dt, *J* = 11.1, 6.1 Hz, 1H), 1.84 (s, 1H). UPLC purity: 100% *m/z*: (pos) 194.39.

2-Chloro-6-((4-chlorophenyl)(1H-tetrazol-5-yl)-methyl)pyridine (52). A solution of 2-(4-chlorophenyl)-2-(6-chloropyridin-2-yl)acetonitrile (309 mg, 1.2 mmol), sodium azide (80 mg, 1.2 mmol), and ammonium chloride (66 mg, 1.2 mmol) in dry DMF (2.4 mL) was heated to 110 °C under Ar for 12 h. The reaction mixture was cooled, diluted with 1 N HCl (1 mL), extracted with ethyl acetate (1 mL x 2) and concentrated. The residue was dissolved in minimal ethyl acetate and precipitated with hexanes to give a crude product. A portion of the crude product (51 mg) was purified by mass-guided HPLC to deliver **52** (12 mg, 3%). ¹H NMR (300 MHz, CDCl₃) δ 7.43 (t, *J* = 7.7 Hz, 1H), 7.01 (d, *J* = 13.2 Hz, 6H), 5.74 (s, 1H). UPLC purity: 100% *m/z*: (pos) 306.32.

5-(1-(4-Chlorophenyl)pentyl)-1H-tetrazole (53). A solution of 2-(4-chlorophenyl)-2-(6-chloropyridin-2-yl)acetonitrile (210 mg, 1.0 mmol), sodium azide (76 mg, 1.2 mmol), and ammonium chloride (62 mg, 1.2 mmol) in dry DMF (2.4 mL) was heated to 110 °C under Ar for 12 h. The reaction mixture was cooled, diluted with 1 N HCl (1 mL), extracted with ethyl

acetate (1 mL x 2) and concentrated. The residue was crystallized from ethyl acetate and hexanes to give **53** as an apparent 3:1 mixture of tautomers (47 mg, 19%). ¹H NMR (300 MHz, CDCl₃) δ 15.86 (s), 7.95 (s), 7.39–7.16 (m), 4.41 (t, *J* = 7.9 Hz), 3.88–3.77 (m), 2.32 (m), 2.21–2.03 (m), 1.89 (m), 1.48–1.13 (m), 0.89 (t, *J* = 7.1 Hz), 0.83 (t, *J* = 7.1 Hz). UPLC purity: 100% *m/z*: (pos) 251.41.

2-(4-Chlorophenyl)-2-(5-(trifluoromethyl)pyridin-2-yl)propanenitrile (54). A solution of 2-chloro-5-(trifluoro-methyl)pyridine (100 mg, 0.55 mmol) and 2-(4-chlorophenyl) acetonitrile (70 μl, 0.55 mmol) in dry DMF (0.5 mL) was added slowly to a solution of potassium *tert*-butoxide (126 mg, 1.1 mmol) in dry DMF (0.5 mL) at 0 °C under Ar. After 10 min, the ice bath was removed and the reaction mixture stirred for 5 h. The reaction was cooled back to 0 °C and iodomethane (82 μl, 1.3 mmol) was added dropwise. After 10 min, the ice bath was removed and reaction mixture was stirred for 2 h. The reaction was quenched over satd aq NH₄Cl (4 mL), extracted with ethyl acetate (4 mL x 2), dried (Na₂SO₄), filtered and concentrated. The crude product was purified by silica flash chromatography with ethyl acetate and hexanes to give **54** (19 mg, 6%). ¹H NMR (300 MHz, CDCl₃) δ 8.89 (s, 1H), 7.95 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 7.48–7.32 (m, 4H), 2.19 (s, 3H). UPLC purity: 100% *m/z*: (pos) 311.22.

8-Chloro-6,11-dihydro-5H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-carbonitrile (55). Prior to use, DME and EtOH were dried over 3Å molecular sieves. 8-chloro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11(6H)-one (246 mg, 1.0 mmol) was dissolved in DME (4 mL) and EtOH (1 mL), then 2-tosylacetonitrile (256 mg, 1.3 mmol, 1.3 equiv) was added and the reaction was cooled to 0 °C. To the solution, potassium *tert*-butoxide (272 mg, 2.4 mmol, 2.4 equiv) was added as a solid. The mixture was stirred for 15 min on ice, warmed to rt over 30 min, and heated to 40 °C for 1 h. The reaction was quenched with water and extracted with

ether. The organic layers were washed with brine, dried over MgSO₄, filtered, and concentrated to dryness. The crude product was purified by flash chromatography on silica gel using 0–20% ethyl acetate in hexanes to deliver nitrile **55** (67 mg, 26%). ¹H NMR (300 MHz, CDCl₃) δ 8.41 (s, 1H), 7.46 (d, *J* = 8.5 Hz, 2H), 7.24 (t, *J* = 7.1 Hz, 3H), 5.64 (s, 1H), 3.45–3.12 (m, 4H). LCMS purity: 95% *m/z*: (pos) 255.19; (neg) 253.20.

Production and Purification of PvdQ

P. aeruginosa PvdQ acylase protein was prepared as previously described by Drake.¹ Briefly, PvdQ was directed to the periplasm of *E. coli* BL21(DE3) and produced without a purification tag.^{1,2} Through standard expression, the protein was processed via autoproteolytic cleavage to the mature α/β heterodimer. The periplasmic proteins were obtained via osmotic lysis with 0.5 M sucrose. PvdQ was then purified via sequential ammonium sulfate precipitation, anion exchange, and gel filtration chromatography.¹ PvdQ was stored in TNT buffer (50 mM Tris-HCl pH 8.0, 50 mM NaCl, and 0.2 mM Tris (2-carboxyethyl) phosphine hydrochloride (TCEP)).

Determination of the structure of PvdQ bound to 3 and 4.

Co-crystals of PvdQ with the inhibitors were grown at 20 °C by hanging drop vapor diffusion utilizing 10–15% PEG 4000, 50–100 mM RbCl, and 50 mM Hepes (pH 7.5) using 0.250 mM of each ligand. Ethylene glycol was used as a cryoprotectant. Data were collected remotely³ for compound **3** at SSRL and for compound **4** with a Rigaku 007HF X-ray generator. Liganded complexes were determined using difference Fourier methods starting from the coordinates of structure 3L94 from the Protein Data Bank with ligand and water molecules removed from the starting model to avoid bias. Iterative model building and refinement were continued to completion for all liganded PvdQ structures using COOT⁴ and refinement with PHENIX⁵ and REFMAC.⁶ Data collection and refinement statistics can be found in Table S1 of the supporting information.

Table S1. Crystallographic Data for PvdQ bound to HTS lead **3** and probe **4**.

	PvdQ + 3	PvdQ + 4
Data Collection		
PDB Code	4K2F	4K2G
Wavelength (Å)	0.97945	1.54187
Resolution (Å)	2.0	2.30
Space Group	C222 ₁	C222 ₁
Unit Cell parameters (Å)		
a	120.3	120.3
b	166.0	165.5
c	94.2	94.0
R _{merge} ^a (%)	8.9 (24.8)	10.8 (42.7)
Completeness ^a	99.2 (98.5)	94.9 (94.6)
I/σ ^a	7.4 (4.2)	8.1 (2.1)
No. observations	235495	1007469
No. reflections	60638	37739
Refinement		
Refinement resolution (Å)	50.7–2.0	15.5–2.3
R-factor ^a (%)	16.8 (19.6)	19.4 (26.4)
R-free ^a (%)	20.6 (23.5)	24.9 (34.1)
Wilson B-value(Å ²)	24.91	27.13
Average B-factor		
Overall (Å ²)	25.18	27.46
Solvent (Å ²)	35.54	31.22
Ligand (Å ²)	23.35	23.31
RMS deviation from ideal		
Bond lengths (Å)	.022	.022
Bond angles (°)	1.96	1.83

^a The value in parentheses represents the statistics within the highest resolution shell

Representative Growth Curves in the Presence and Absence of EDDHA

As described in the main text, cells were grown in SM9 media and CAA media for various growth and inhibition assays.

SM9 Minimal Media

6.0 g L⁻¹ K₂HPO₄
3.0 g L⁻¹ KH₂PO₄
1.0 g L⁻¹ (NH₄)₂SO₄
0.2 g L⁻¹ MgSO₄
4 g L⁻¹ Succinic Acid
pH to 7 with KOH

CAA Media

5 g L⁻¹ Casamino Acid growth powder
1.54 g L⁻¹ K₂HPO₄
0.25 g L⁻¹ MgSO₄

To validate target specificity prior to initiation of SAR studies, hit compound **3** was examined for effectiveness against *P. aeruginosa* in the presence and absence of EDDHA in minimal media. During growth in minimal media, even in the absence of the chelator, pyoverdine production still improves cell growth due to low iron concentrations. Therefore, we also examined the effect of the compound on a *pvdQ* mutant strain of *P. aeruginosa*. The results show that **3** is effective against cells in the presence or absence of the chelator, although more effective when 2 μM EDDHA is added and the requirement for pyoverdine production is enhanced. The compound shows no impact on the *pvdQ* mutant.

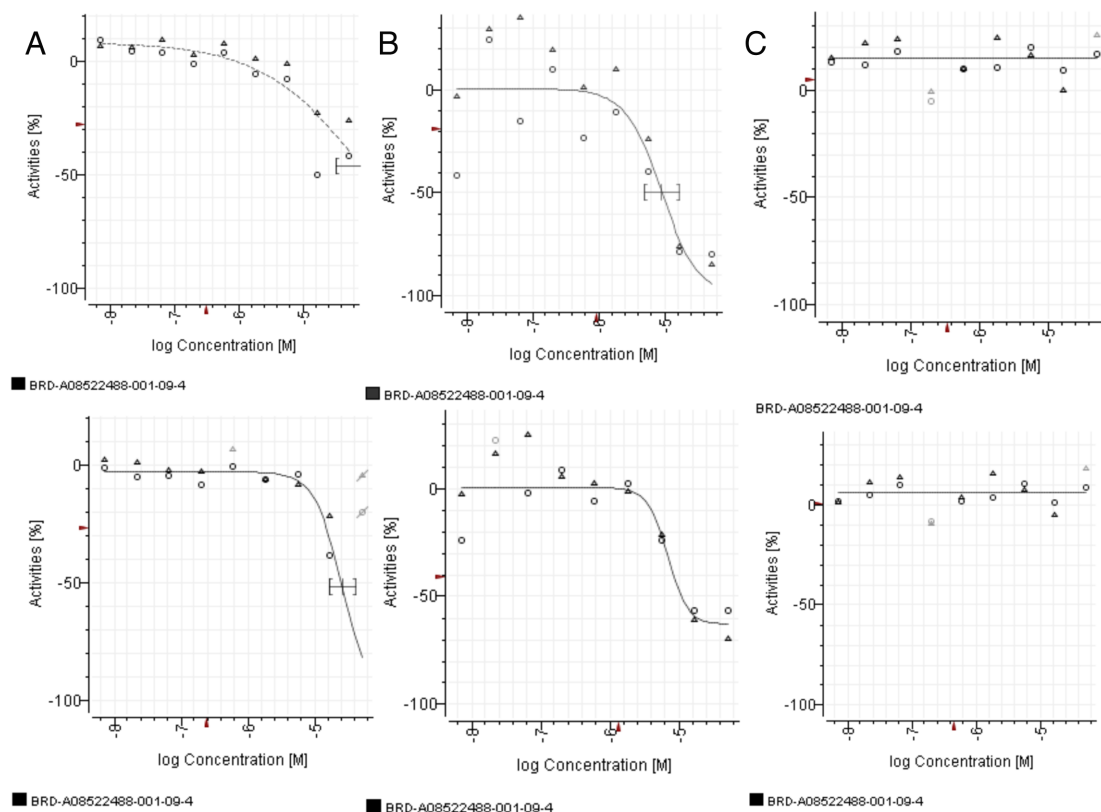


Figure S1. Wild-type *P. aeruginosa* growth curves A. in minimal media in the absence of EDDHA, B. in minimal media in the presence of 2 μM EDDHA, or C. the mutant *pvdQ* strain in the absence of EDDHA. The top panels are monitoring pyoverdine production at 405 nm while the bottom panels monitor cell growth at 605. The % activity is presented as absorbance of treated cells over the absorbance of untreated. Cells were diluted to an OD_{600} of 0.000005 and grown 18-30 h in 40 μl minimal media in a 384 well plate.

Representative Biochemical Inhibition Assay

Subsequently, the final probe compound has been investigated in the biochemical and growth assays described in the text. Multiple assays were performed with different synthetic preparation of **4**, giving minor differences in values largely due to the instability of the compounds described in the main text and below. A representative analysis of the ability of **4** to inhibit the biochemical assay with 4MU-laurate is shown below. Two representative experiments are shown, giving IC_{50} values of 0.005 and 0.011 μM , with Hill coefficients of 0.84 and 0.96, respectively. Two additional assays were performed and deposited with PubChem, giving IC_{50} values of 0.003 and

0.005 μM , that could not be fit to the Hill equation. The raw data are available for all of these assays at: <http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?aid=623985>

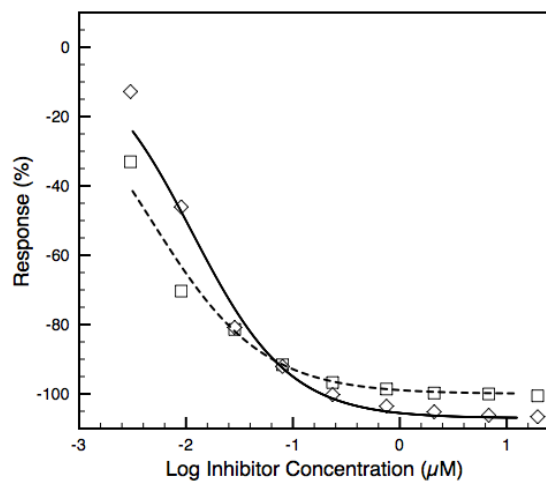


Figure S2. Representative Inhibition plots of PvdQ biochemical assay with Probe Compound 4.

HPLC Based Assay to Detect Pyoverdine Formation

As described in the text, we treated whole cells with the PvdQ inhibitors and monitored pyoverdine production directly by HPLC. Conditioned media was harvested after 4h of treatment with the inhibitors and normalized to cell density. Conditioned medium was subjected to fractionation utilizing HPLC on an Agilent 1260 system in-line with a C18 column (Eclipse 4.6x100 mm, 3.5 μ M) running an aqueous mobile phase containing 0.1% (v/v) formic acid at a flow rate of 1 ml/min. All runs were monitored at 375 nm with the samples reconstituted in the initial HPLC gradient buffer. 25 μ L conditioned media were loaded and runs conducted with a linear gradient from 2% ACN to 64% ACN over 10 minutes at a column temp of 30 $^{\circ}$ C. Peaks with retention times of 3.1 - 3.8 minutes were integrated and summed using the Agilent Chemstation software package, and normalized for cell density. Pyoverdine production was compared to that of untreated cells. A representative HPLC trace is shown below, as well as representative curves depicting responses to the inhibitor **3**, **4**, and **16**.

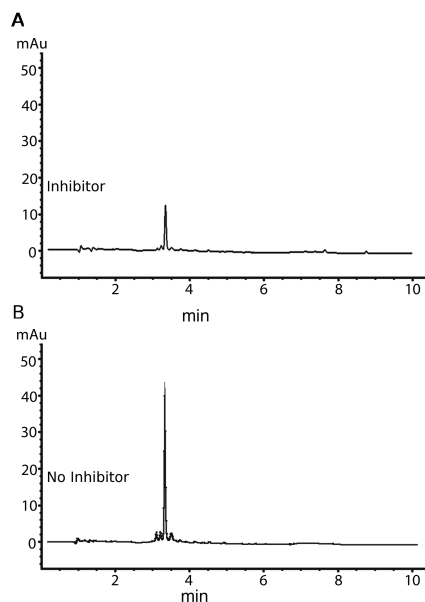


Fig S3. Representative HPLC traces for whole cell IC₅₀ calculations. A) Conditioned media HPLC trace at 375 nm for sample treated with 50 μ M Compound 4 (ML318). B) Conditioned media HPLC trace at 375 nm for an untreated sample.

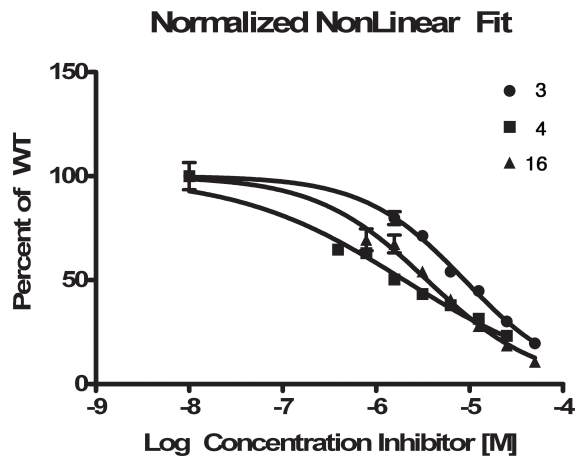


Figure S4. Representative examples of the effect of inhibitor 3, 4, and 16 on pyoverdine production.

PBS Stability. Stability was determined in the presence of PBS, pH 7.4 with 1% DMSO. Each compound was prepared in triplicate at 1 μ M on six separate plates and allowed to equilibrate at room temperature with a 350 rpm orbital shake for 48 h. One plate was removed at each time point (0, 2, 4, 8, 24, and 48 h). An aliquot was removed from each well and analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer. Additionally, to the remaining material at each time point, methanol was added to force dissolution of compound (to test for recovery of compound). An aliquot of this was also analyzed by UPLC-MS.

Compound	T0	T2	T4	T8	T24	T48
antipyrine	100	91	86	86	92	91
unstable control	100	44	4	20	5	1
compound 3	100	75	49	34	32	39
compound 4	100	92	70	65	46	50

GSH Stability. Stability was determined in the presence of PBS pH 7.4 μ M and 50 μ M glutathione with 1% DMSO. Each compound was prepared in duplicate at 1 μ M on six separate plates and allowed to equilibrate at room temperature with a 350 rpm orbital shake for 6 h. One plate was removed at each time point (0, 0.5, 1, 2, 4, and 6 h). An aliquot was removed from each well and analyzed by UPLC-MS (Waters, Milford, MA) with compounds detected by SIR detection on a single quadrupole mass spectrometer. Additionally, to the remaining material at each time point, methanol was added to force dissolution of compound (to test for recovery of compound). An aliquot of this was also analyzed by UPLC-MS.

Compound	T0	T0.5	T1	T2	T4	T6
antipyrine	100.0	99	101	98	85	91
ethacrynic Acid	100.0	63	64	52	35	34
compound 3	100.0	92	90	59	88	90
compound 4	100.0	102	90	80	91	227

PAK Mutant Strain Construction

To construct the *mexAB-oprM* deletion, ~1 kb chromosomal DNA segments flanking the targeted locus were PCR amplified from PAK chromosomal DNA by using specific oligonucleotide primers (Table S1) and were joined through splicing by overlap extension-PCR;⁷ deletions were marked by inclusion of a unique *Hind*III restriction site in the overlapping (internal) primers. The resulting ~2 kb DNA fragment was inserted into the KAN^r Gateway entry vector pDONR201 (Gateway cloning system; Invitrogen, Carlsbad, CA) and transferred into the Gateway destination vector pEXGmGW⁸ resulting in pEXGmGWD- Δ *mexAB-oprM*. This suicide plasmid was introduced into *P. aeruginosa* PAK by electroporation, selected for chromosomal insertion on LB agar containing gentamicin (100 μ g/mL), and then counter-selected for loss of the plasmid backbone on LB agar containing 5% sucrose.⁹ To confirm deletions, the region surrounding the target gene was PCR amplified from chromosomal DNA and digested with the *Hind*III to detect the unique marker.

Table S2. Oligonucleotide primers for construction of Δ *mexABoprM*

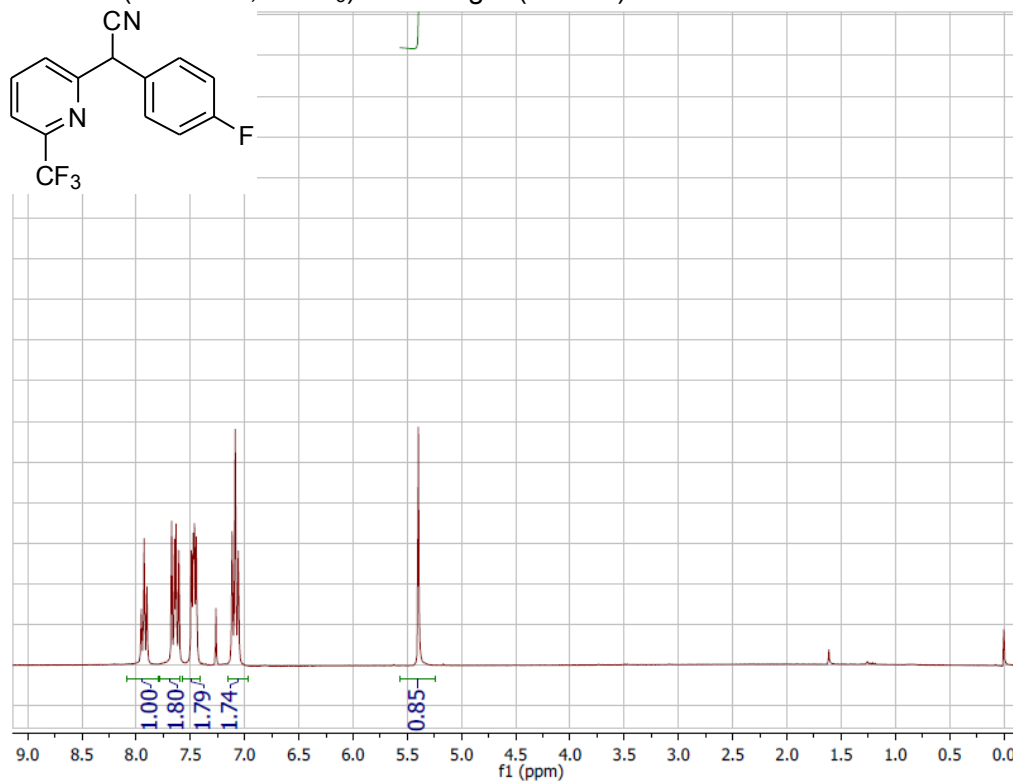
Primer Name	Primer No.	Sequence (5' to 3')
Δ <i>mexAB-oprM</i> - 5' flanking	SM 1054	ACCAACCATGCCGAGCGTG
Δ <i>mexAB-oprM</i> - 5' delete (F) + attB1	SM 1055	GGGGACAAGTTTGTACAAAAAAGCAGGCTC AAGCCGGTCACCATCAAGG
Δ <i>mexAB-oprM</i> - 5' delete (R)	SM 1060	CGATCAAGCCTGGGGATCTTCAAGCTTGGC TGGCGTTCGTTGCATAGC
Δ <i>mexAB-oprM</i> - 3' delete (F)	SM 1061	GCTATGCAACGAACGCCAGCCAAGCTTGAA GATCCCCAGGCTTGATCG
Δ <i>mexAB-oprM</i> - 3' delete (R) + attB2	SM 1062	GGGGACCACTTTGTACAAGAAAGCTGGGTG ACCAGTTGCCCCATGTGG
Δ <i>mexAB-oprM</i> - 3' flanking	SM 1063	GATCCATGGCAACAAGAGCC

Supporting References

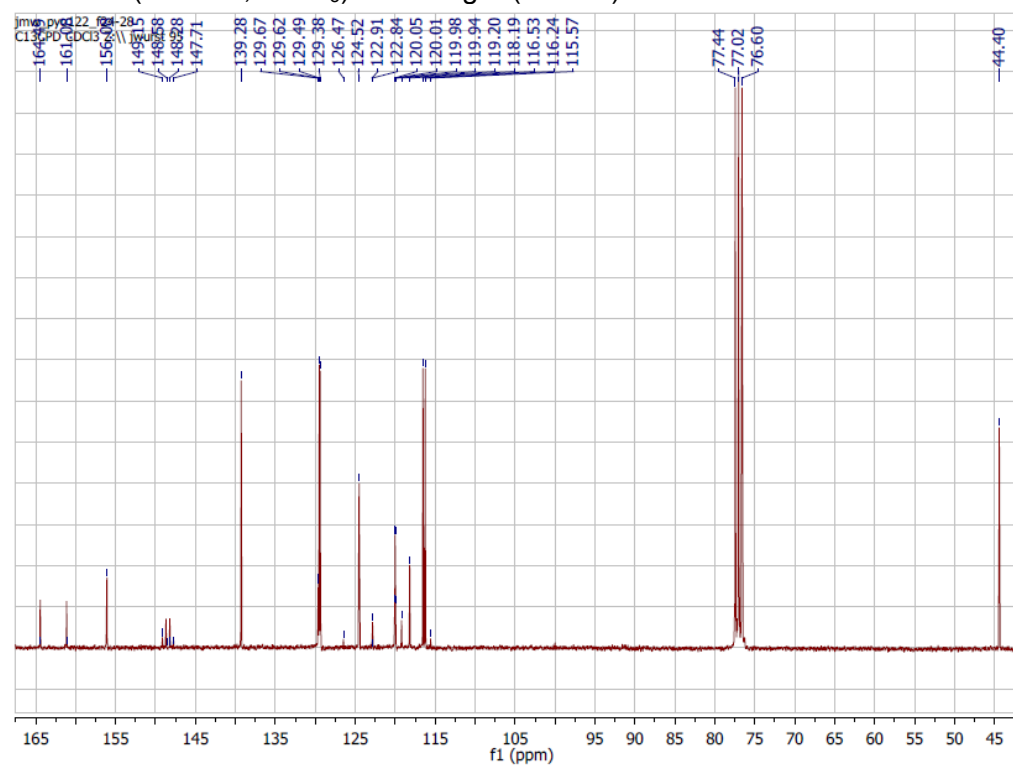
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- (8) Wolfgang, M. C.; Lee, V. T.; Gilmore, M. E.; Lory, S. *Developmental cell* **2003**, *4*, 253.
- (9) Hoang, T. T.; Karkhoff-Schweizer, R. R.; Kutchma, A. J.; Schweizer, H. P. *Gene* **1998**, *212*, 77.

NMR Spectra of Table 6 Compounds

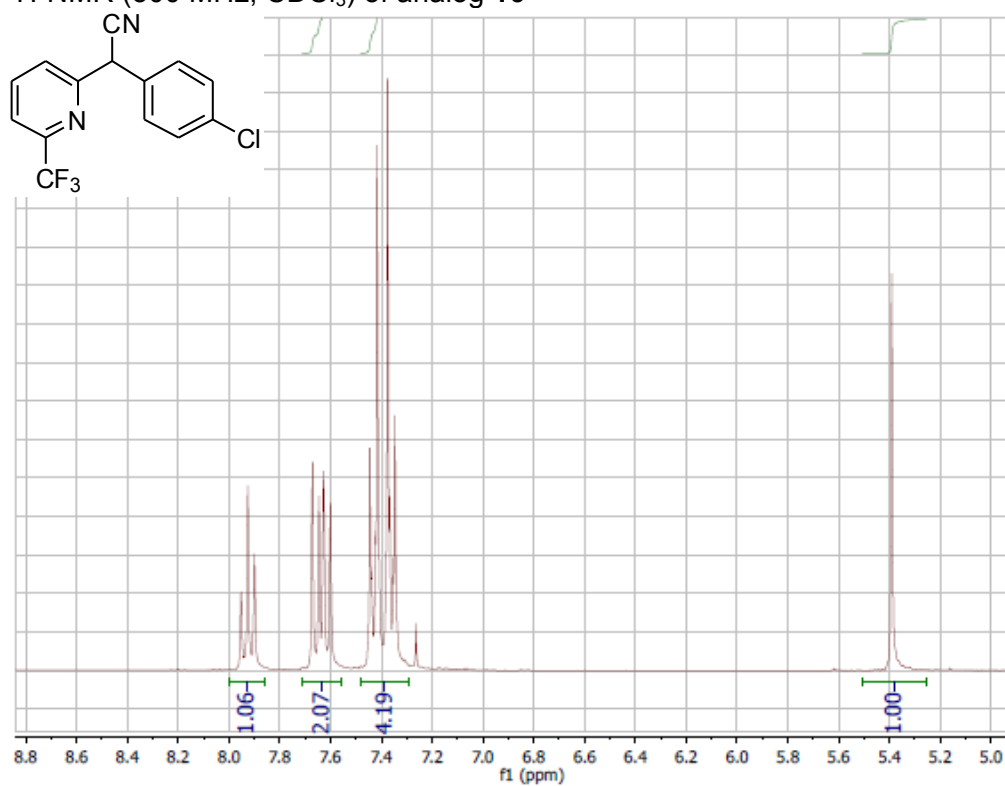
¹H-NMR (300 MHz, CDCl₃) of analog **4** (ML318)



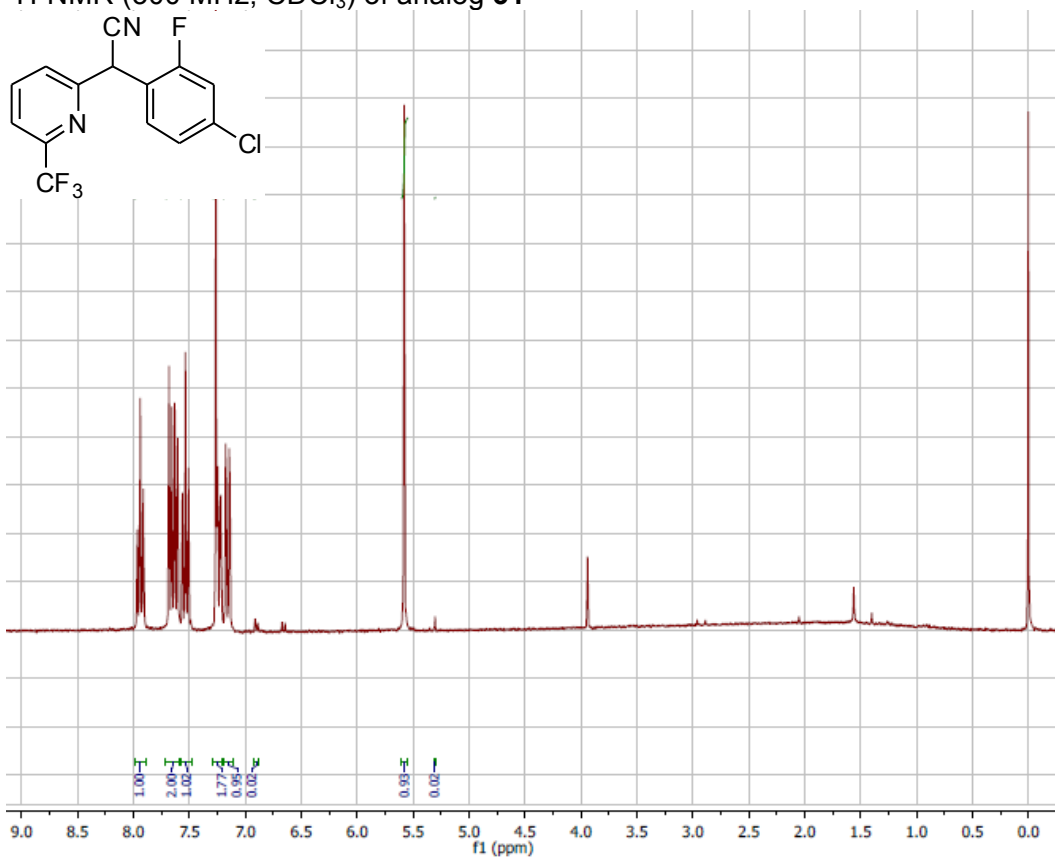
¹³C-NMR (75 MHz, CDCl₃) of analog **4** (ML318)



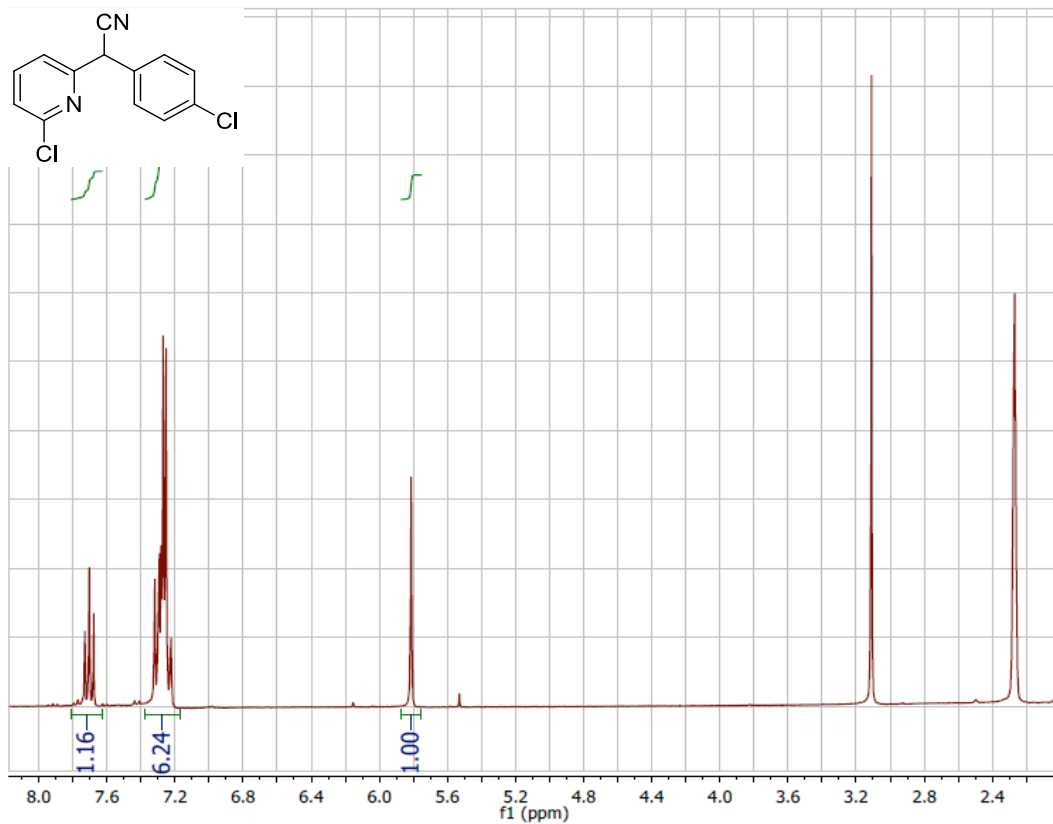
¹H-NMR (300 MHz, CDCl₃) of analog **16**



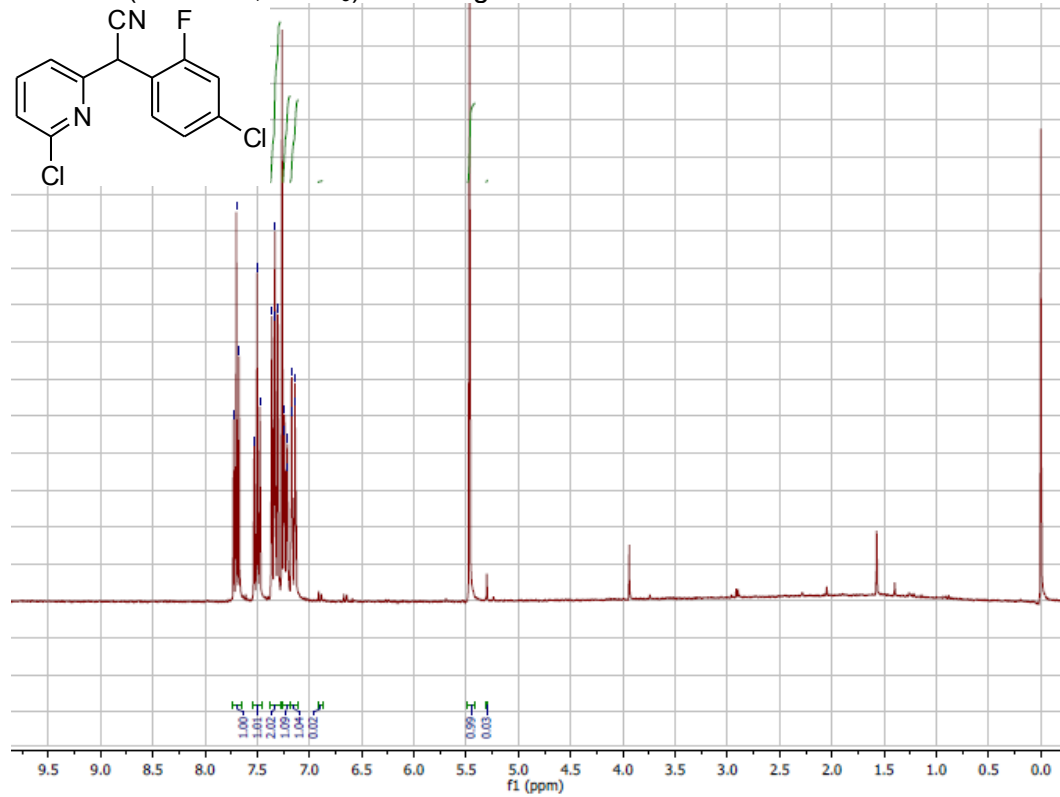
¹H-NMR (300 MHz, CDCl₃) of analog **31**



¹H-NMR (300 MHz, CDCl₃) of analog 3



¹H-NMR (300 MHz, CDCl₃) of analog 13



¹H-NMR (300 MHz, CDCl₃) of analog **15**

