Supplementary Materials

Evaluation of Heterocyclic Carboxamides as Potential Efflux Pump Inhibitors in *Pseudomonas aeruginosa*

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Chemistry Supporting Information

All solvents and chemicals were used as purchased without further purification. Mass spectrometry (MS) was performed by electrospray (ESI) ionization using a Shimadzu 2020 LC-MS quadrupole mass spectrometer. ^{1}H and ^{13}C-NMR were recorded using a Varian Oxford 300, Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak for ^{1}H and ^{13}C). The coupling constants (*J*) are reported in hertz (Hz).

All new compounds are prepared according to the general synthetic procedure and the spectral data is reported here:

(*S*)-*N*-(2,5-diaminopentyl)-5-(4-fluorophenyl)benzo[*d*]thiazole-2-carboxamide hydrogen chloride salt **6a**.

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.32 (m, 1H), 8.30 (m, 1H), 8.29 (m, 1H), 8.22 (s br, 2H), 8.05 (s br, 2H), 7.88 (m, 1H), 7.83 (m, 2H), 7.35 (t, *J* = 9 Hz, 2H), 3.50-3.68 (m, 3H), 2.79 (m, 2H), 1.68 (m, 4H).

(*S*)-*N*-(2,5-diaminopentyl)-6-(4-fluorophenyl)benzo[*d*]thiazole-2-carboxamide hydrogen chloride salt **6b**.

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.31 (m, 1H), 8.53 (s, 1H), 8.28 (s br, 2H), 8.16 (m, 1H), 8.13 (s br, 2H), 7.90 (m, 1H), 7.81 (m, 2H), 7.34 (t, *J* = 9 Hz, 2H), 3.46-3.66 (m, 3H), 2.79 (m, 2H), 1.69 (m, 4H). MS (ESI+) m/z: calcd for $C_{19}H_{22}FN_4OS$ $[M+H]^+$: 373.15; found 373.20.

(*S*)-*N*-(2,5-diaminopentyl)-6-(4-fluorophenyl)-1*H*-benzo[*d*]imidazole-2-carboxamide hydrogen chloride salt **6c** has been previously described by E. J. LaVoie et al. in patent application: WO2018,165,611.

(*R*)-*N*-(2,5-diaminopentyl)-6-(4-fluorophenyl)-1*H*-benzo[*d*]imidazole-2-carboxamide hydrogen chloride salt **6d** has been previously described by E. J. LaVoie et al. in patent application: WO2018,165,611.

(*S*)-*N*-(2,5-diaminopentyl)-5-(4-fluorophenyl)benzofuran-2-carboxamide hydrogen chloride salt **6e**

^{1}H NMR (300 MHz, D_2O) δ 8.23 (s, 1H), 7.71 (m, 3H), 7.66 (m, 2H), 7.20 (t, *J* = 9 Hz, 2H), 3.75 (m, 1H), 3.67 (m, 1H), 3.57 (m, 1H), 3.02 (m, 2H), 1.82 (m, 4H). MS (ESI+) m/z: calcd for $C_{20}H_{23}FN_3O_2$ $[M+H]^+$: 356.18; found 356.05.

(*S*)-*N*-(2,5-diaminopentyl)-6-(4-fluorophenyl)benzofuran-2-carboxamide hydrogen chloride salt **6f**

^{1}H NMR (300 MHz, D_2O) δ 7.81 (m, 2H), 7.72 (m, 2H), 7.61 (m, 1H), 7.55 (s, 1H), 7.24 (t, *J* = 9 Hz, 2H), 3.78 (m, 1H), 3.63 (m, 1H), 3.58 (m, 1H), 3.05 (m, 2H), 1.83 (m, 4H). MS (ESI+) m/z: calcd for $C_{20}H_{23}FN_3O_2$ $[M+H]^+$: 356.18; found 356.05.

(*S*)-*N*-(2,5-diaminopentyl)-5-(4-fluorophenyl)benzo[*b*]thiophene-2-carboxamide hydrogen chloride salt **6g**

^{1}H NMR (300 MHz, D_2O) δ 7.97 (s, 1H), 7.85 (m, 1H), 7.78 (s, 1H), 7.60 (m, 3H), 7.18 (m, 2H), 3.66 (m, 1H), 3.61 (m, 1H), 3.52 (m, 1H), 3.06 (m, 2H), 1.81 (m, 4H). MS (ESI+) m/z: calcd for $C_{20}H_{23}FN_3OS$ $[M+H]^+$: 372.15; found 372.00.

(*S*)-*N*-(2,5-diaminopentyl)-5-(4-fluorophenyl)benzo[*b*]thiophene-2-carboxamide hydrogen chloride salt **6h**

^{1}H NMR (300 MHz, D_2O) δ 7.96 (s, 1H), 7.84 (m, 1H), 7.82 (s, 1H), 7.60 (m, 3H), 7.18 (m, 2H), 3.64 (m, 1H), 3.60 (m, 1H), 3.51 (m, 1H), 3.04 (m, 2H), 1.81 (m, 4H). MS (ESI+) m/z: calcd for $C_{20}H_{23}FN_3OS$ $[M+H]^+$: 372.15; found 372.00.

(*S*)-*N*-(2,5-diaminopentyl)-5-(4-fluorophenyl)-1*H*-indole-2-carboxamide hydrogen chloride salt **6i** has been previously described by E. J. LaVoie et al. in patent application: WO2018,165,612.

(*R*)-*N*-(2,5-diaminopentyl)-5-(4-fluorophenyl)-1*H*-indole-2-carboxamide hydrogen chloride salt **6k** has been previously described by E. J. LaVoie et al. in patent application: WO2018,165,612.

(*R*)-*N*-(2,5-diaminopentyl)-6-(4-fluorophenyl)-1*H*-indole-2-carboxamide hydrogen chloride salt **6l** has been previously described by E. J. LaVoie et al. in patent application: WO2018,165,612

(*S*)-*N*-(2,5-diaminopentyl)-5-(4-fluorophenyl)-1*H*-pyrrolo[2,3-*c*]pyridine-2-carboxamide hydrogen chloride salt **6m**

^{1}H NMR (300 MHz, CD_3OD) δ 9.07 (s, 1H), 8.48 (s, 1H), 7.93 (m, 2H), 7.71 (s, 1H), 7.40 (t, *J* = 8.7 Hz, 2H), 3.83 (m, 1H), 3.68 (m, 1H), 3.52 (m, 1H), 3.04 (m, 2H), 1.89 (m, 4H). ^{13}C NMR (75 MHz, CD_3OD) δ 161.02, 143.04, 140.24, 138.53, 130.96, 129.94, 129.83, 129.74, 129.19, 128.47, 117.25, 116.40, 116.09, 104.17, 51.81, 40.63, 38.85, 27.14, 23.23. MS (ESI+) m/z: calcd for $C_{19}H_{23}FN_5O$ $[M+H]^+$: 356.19; found 356.20.

(*S*)-*N*-(2,5-diaminopentyl)-6-(4-fluorophenyl)-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxamide hydrogen chloride salt **6n**

^{1}H NMR (300 MHz, D_2O) δ 9.14 (s, 1H), 7.88 (s, 1H), 7.85 (m, 2H), 7.55 (s, 1H), 7.36 (t, *J* = 8.7 Hz, 2H), 3.82 (m, 1H), 3.69 (m, 1H), 3.59 (m, 1H), 3.04 (m, 2H), 1.82 (m, 4H). MS (ESI+) m/z: calcd for $C_{19}H_{23}FN_5O$ $[M+H]^+$: 356.19; found 356.25.

Table S1. Levofloxacin potentiation in the presence of EPI on *P. aeruginosa* strains overexpressing efflux pumps.

Compound	Strain				
	K767 (WT)	K1455* (↑mexAB-oprM)	K2415* (↑mexXY-oprM)	K2951* (↑mexCD-oprJ)	K2376# (↑mexEF-oprN)
	Levofloxacin MIC (μg/mL), (fold difference)				
6f	0.125, (4)	1, (4)	0.5, (4)	0.125, (4)	4, (2)
6g	0.063, (8)	0.25, (16)	0.25, (8)	0.063, (8)	0.5, (16)
6h	0.25, (2)	1, (4)	0.5, (4)	0.125, (4)	4, (2)
6i	0.25, (2)	1, (4)	0.5, (4)	0.25, (2)	2, (4)
6j (TXA01182)	0.063, (8)	0.5, (8)	0.25, (8)	0.125, (4)	2, (4)
6l	0.125, (4)	0.5, (8)	0.25, (8)	0.125, (4)	2, (4)
-	0.5	4	2	0.5	8

* Derived from K767, # clinical isolate.

Table S2. Ciprofloxacin and tigecycline potentiation in the presence of TXA01182 on resistant clinical isolates.

P. aeruginosa Strain	Ciprofloxacin MIC (μg/mL), (fold difference)		Tigecycline MIC (μg/mL), (fold difference)		Resistance Mechanisms
	No EPI	+ TXA01182 (6.25 μg/mL)	No EPI	+ TXA01182 (6.25 μg/mL)	
AR-0229	32	8, (4)	128	8, (16)	*gyrA-T83I, nalC-G71E, mexR-V126Q*
AR-0232	4	1, (4)	16	8, (2)	*gyrA-T83I, nalC-G71E, mexR-V126Q*
AR-0234	4	1, (4)	16	8, (2)	*gyrA-T83I, nalC-G71E, mexR-V126Q*
AR-0239	32	16, (2)	16	8, (2)	*gyrA-T83I, nalC-G71E, mexR-V126Q*
AR-0244	64	16, (4)	64	8, (8)	*gyrA-T133H, nalC-G71E, mexR-V126Q*
AR-0246	32	16, (2)	64	16, (4)	*gyrA-T83I ,nalC-G71E, mexR-V126Q*
AR-0249	32	8, (4)	64	4, (16)	*gyrA-T83I, nalC-G71E*
AR-0264	32	8, (4)	16	2, (8)	*gyrA-D87Y, nalC-G71E*

Table S3. Frequency of resistance to Cefepime alone and in combination with TXA01182.

Strain	Cefepime alone (4 μg/mL)	Cefepime (4 μg/mL) + TXA01182 (12.5 μg/mL)
P. aeruginosa ATCC 27853	2.05×10^{-6}	$<1.04 \times 10^{-7}$