

**IC50 determination of OSA compounds as *P. aeruginosa* MurC and MurF inhibitors received
in May and July, 2023 (Yiwei)**

and

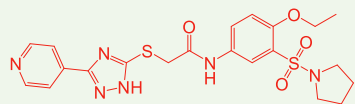
Side by side comparison of all IC50s for all Mur ligases challenged with this compound set.

Adrian Lloyd, Laura Diaz Saez, Julie Tod and Christopher Dowson

8th May, 2024

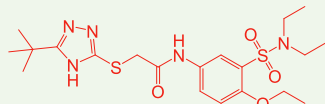
OSA Compounds at 0.5 mM sent by Yiwei Wang on the 23rd May and 25th July, 2023 found to exert >50% inhibition of *P. aeruginosa* MurC activity, colour coded relative to multiple Mur ligase targeting.

98.66 ± 1.02



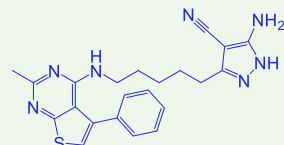
OSA_001147

97.38 ± 1.47



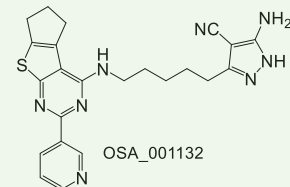
OSA_001145

96.29 ± 2.04



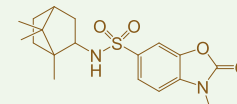
OSA_001133

96.12 ± 1.50



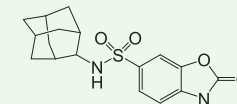
OSA_001132

92.73 ± 2.56



OSA_001167

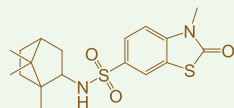
92.26 ± 10.00



OSA_001159

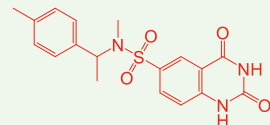
Mean ± SD (n=3) of percentage inhibition relative to vehicle (DMSO) shown above each structure.

91.91 ± 7.38



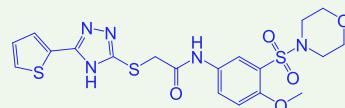
OSA_001160

91.12 ± 1.33



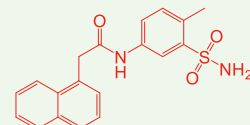
OSA_001164

88.08 ± 1.17



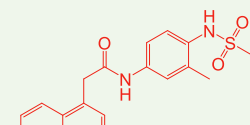
OSA_001148

86.36 ± 2.21



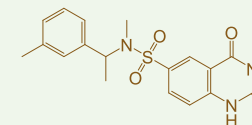
OSA_001156

86.16 ± 2.56



OSA_001155

84.76 ± 4.99



OSA_001168

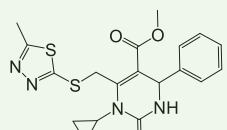
Red structures: MurC inhibitors with >50% potency vs MurD and E

Blue structures: MurC inhibitors with > 50% potency vs MurE

Brown structures: MurC inhibitors with >50% potency vs MurD.

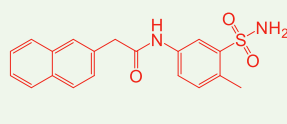
OSA_descriptors below each structure as supplied.

83.75 ± 2.04



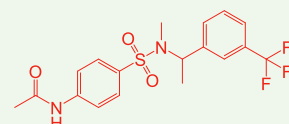
OSA_001136

83.66 ± 3.47



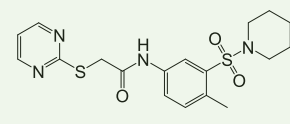
OSA_001153

82.82 ± 2.54



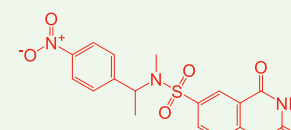
OSA_001169

82.41 ± 0.98



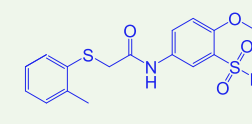
OSA_001141

81.51 ± 1.68



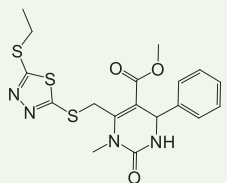
OSA_001172

76.69 ± 2.75



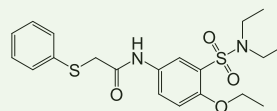
OSA_001149

75.71 ± 6.33



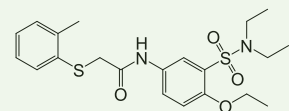
OSA_001134

68.73 ± 5.65



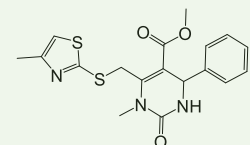
OSA_001150

63.28 ± 8.09



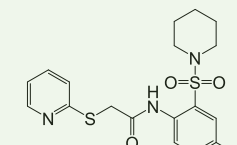
OSA_001151

63.24 ± 4.05



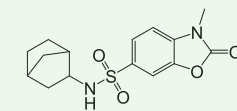
OSA_001135

61.81 ± 3.61



OSA_001142

53.55 ± 7.96



OSA_001157

Characterization of inhibition of *P. aeruginosa* MurC by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Methodology

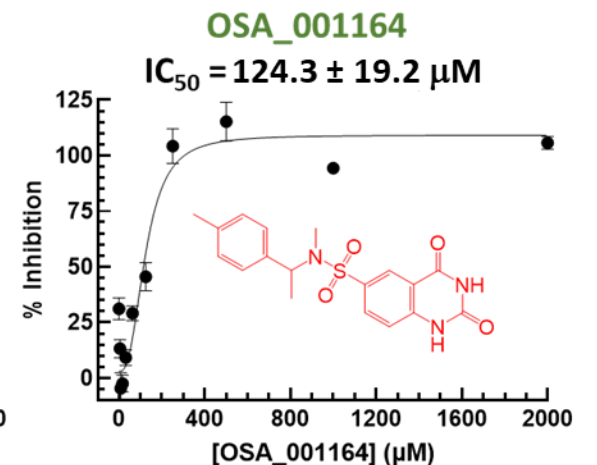
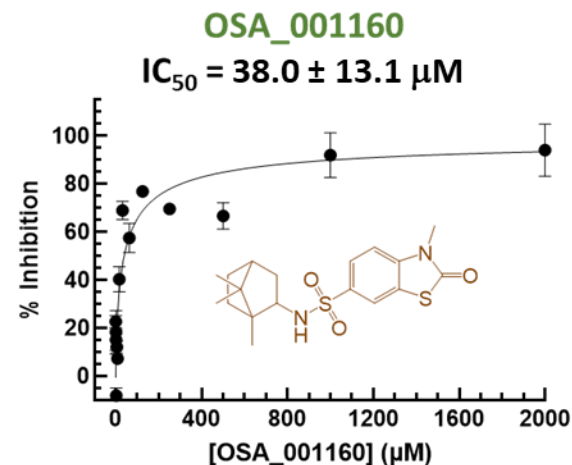
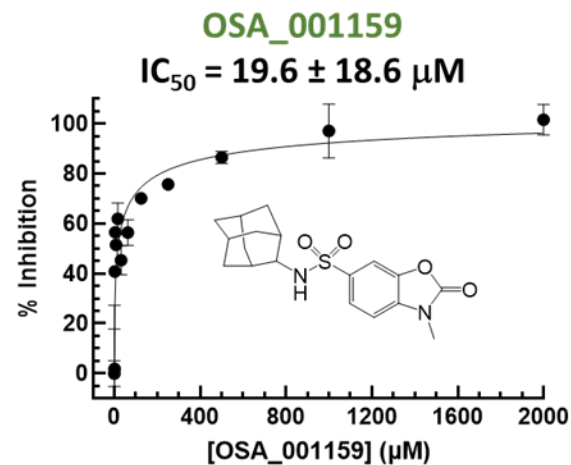
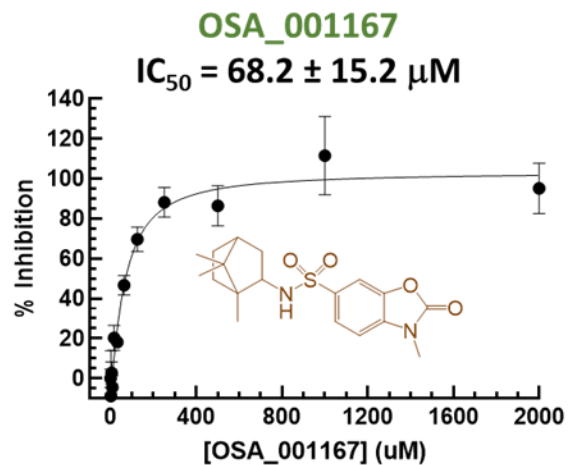
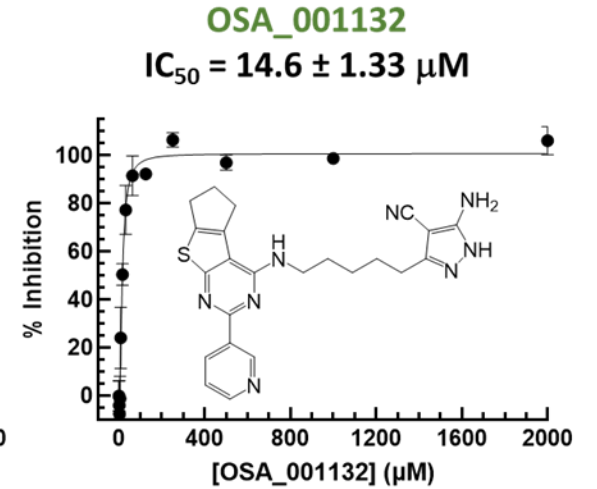
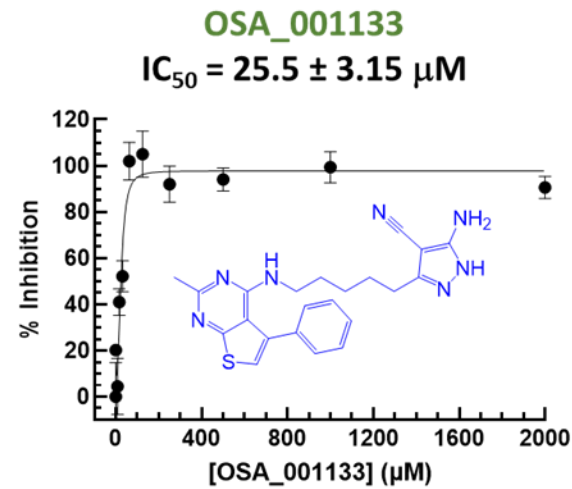
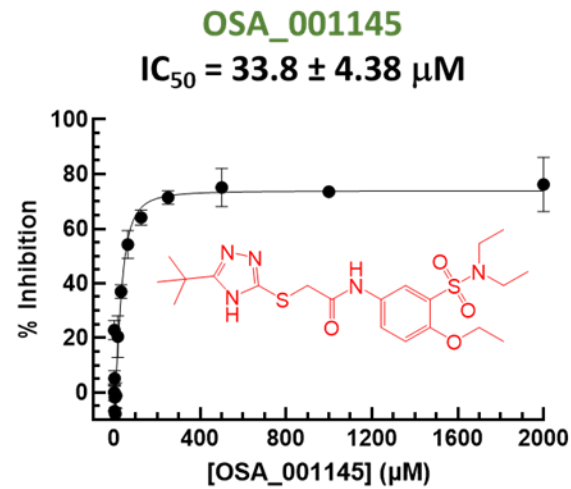
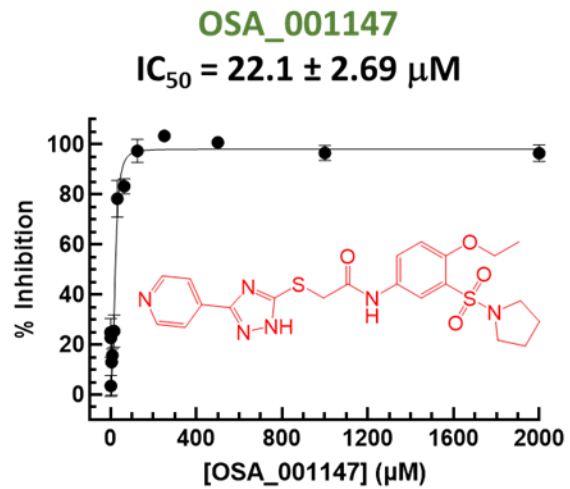
MurC Assay:

Assays were performed at 30°C in a 10 µl volume/384 well format, containing 50 mM MOPS, 10 mM MgCl₂, 0.5 mM inosine, 2.5 mM.min⁻¹ *Arthrobacter* sp. xanthine oxidase, 20 mM.min⁻¹ horse radish peroxidase, 50 µM amplex Red, 2.64 mM.min⁻¹ *E. coli* purine nucleoside phosphorylase, 60 µM ATP (where added), 0.1 mM UDP-MurNAc, 15.1 nM *P. aeruginosa* MurC and 5mM L-alanine. Compounds were added from 50 mM stocks in DMSO, where the final concentrations of DMSO and compound were 1.16 % (v/v) and in the range 0.49 to 2000 µM respectively. Controls without compound contained 1.16% (v/v) DMSO. Per compound/DMSO/ADPCP concentration, MurC was assayed in three wells where reaction was initiated by addition of ATP and in three wells where reaction (control) was initiated by water. The fluorescent product of the reaction cascade (resorufin – derived from amplex red) was continuously monitored from above the well at an excitation and emission wavelength of 545 nm and 585 nm respectively in a Varioskan Lux plate reader. Percentage inhibition was related to compound concentration using a four parameter model from the standard equation menu in GraphPad Prism 9.1 to yield estimates of IC₅₀ values and Hill coefficients.

Characterization of inhibition of *P. aeruginosa* MurC by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Complete Data Set

Compound	IC50 ($\mu\text{M} \pm \text{SD}$) (n = 3)	Hill Coefficient	Original Hit % Inhibition at 0.5 mM
ADPCP	0.38 \pm 0.04	1.50 \pm 0.23	95.5 \pm 1.98
OSA_001147	22.1 \pm 2.69	2.67 \pm 0.73	98.7 \pm 1.02
OSA_001145	33.8 \pm 4.38	1.81 \pm 0.37	97.4 \pm 1.47
OSA_001133	25.5 \pm 3.15	2.52 \pm 0.67	96.3 \pm 2.04
OSA_001132	14.6 \pm 1.33	1.73 \pm 0.24	96.1 \pm 1.50
OSA_001167	68.2 \pm 15.2	1.24 \pm 0.31	92.7 \pm 2.56
OSA_001159	19.6 \pm 18.6	0.45 \pm 0.14	92.3 \pm 10.0
OSA_001160	38.0 \pm 13.1	0.67 \pm 0.13	91.9 \pm 7.38
OSA_001164	124.3 \pm 19.2	2.63 \pm 0.96	91.1 \pm 1.33
OSA_001148	130.9 \pm 30.2	1.04 \pm 0.20	88.1 \pm 1.17
OSA_001156	244.5 \pm 41.3	1.21 \pm 0.22	86.4 \pm 2.21
OSA_001155	58.00 \pm 6.20	1.74 \pm 0.29	86.2 \pm 2.56
OSA_001168	127.4 \pm 17.2	1.82 \pm 0.39	84.8 \pm 4.99
OSA_001136	238.6 \pm 114.7	0.62 \pm 0.16	83.8 \pm 2.04
OSA_001153	299.1 \pm 95.07	1.00 \pm 0.18	83.7 \pm 3.47
OSA_001169	25.37 \pm 3.74	1.48 \pm 0.29	82.8 \pm 2.54
OSA_001141	729.9 \pm 171.8	0.58 \pm 0.08	82.4 \pm 0.98
OSA_001172	73.82 \pm 182.9	0.31 \pm 0.11	81.5 \pm 1.68
OSA_001149	140.1 \pm 27.54	0.77 \pm 0.10	76.7 \pm 2.75
OSA_001134	132.0 \pm 23.54	1.04 \pm 0.19	75.7 \pm 6.33
OSA_001150	184.0 \pm 25.6	1.00 \pm 0.12	68.7 \pm 5.65
OSA_001151	80.00 \pm 93.02	0.38 \pm 0.10	63.3 \pm 8.09
OSA_001135	422.3 \pm 157.2	1.21 \pm 0.33	63.2 \pm 4.05
OSA_001142	109.8 \pm 17.5	0.96 \pm 0.13	61.8 \pm 6.61
OSA_001157	423.9 \pm 66.02	1.27 \pm 0.24	53.55 \pm 7.96

Characterization of inhibition of *P. aeruginosa* MurC by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Dose Response Curves (1)

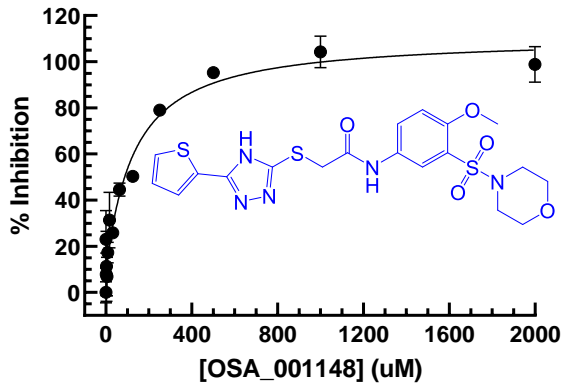


Red structures: MurC inhibitors with >50% potency vs MurD and E; Blue structures: MurC inhibitors with >50% potency vs MurE;
Brown structures: MurC inhibitors with >50% potency vs MurD.

Characterization of inhibition of *P. aeruginosa* MurC by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Dose Response Curves (2)

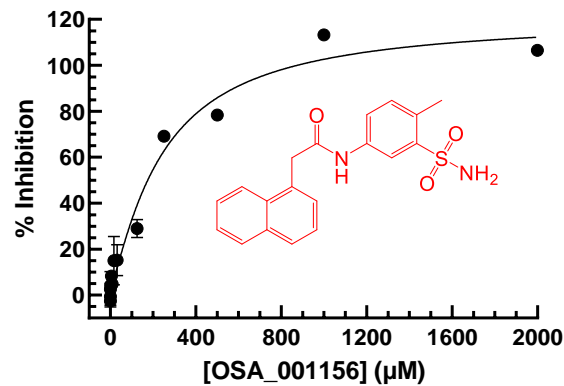
OSA_001148

IC50 = 130.9 ± 30.2 μM



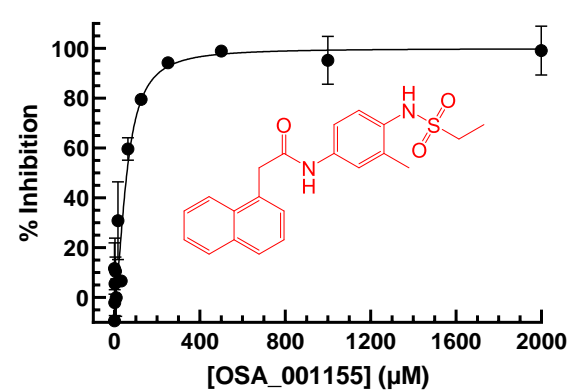
OSA_001156

IC50 = 244.5 ± 41.3 μM



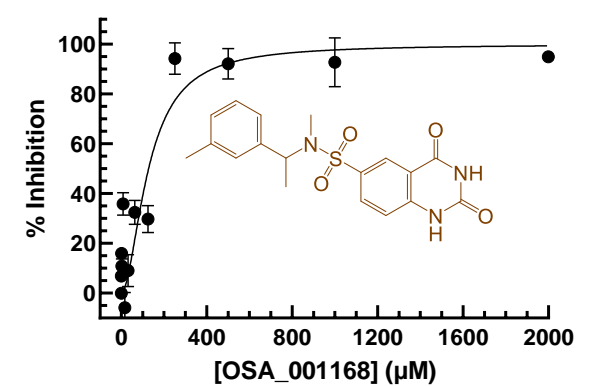
OSA_001155

IC50 = 58.00 ± 6.20 μM



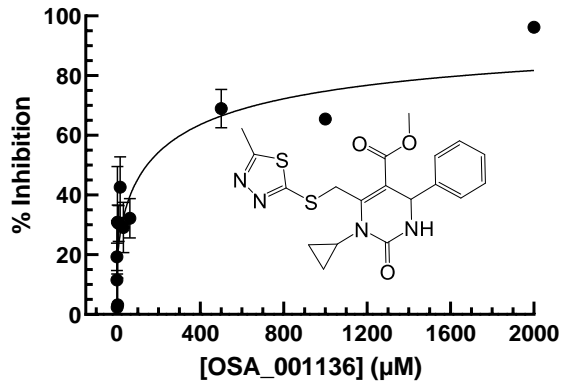
OSA_001168

IC50 = 127.4 ± 17.2 μM



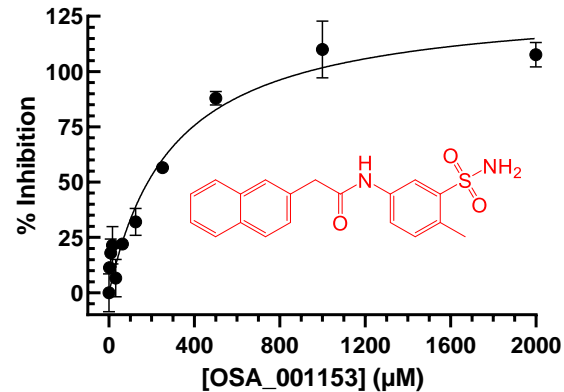
OSA_001136

IC50 = 238.6 ± 114.7 μM



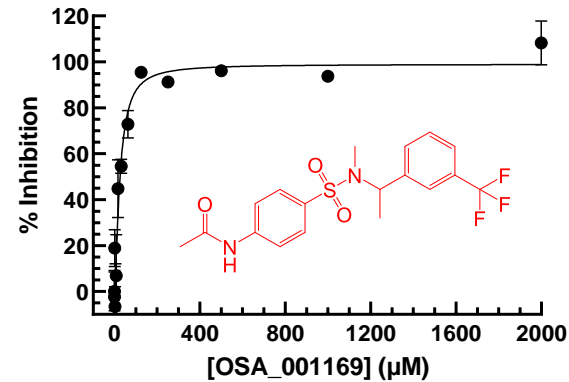
OSA_001153

IC50 = 299.1 ± 95.07 μM



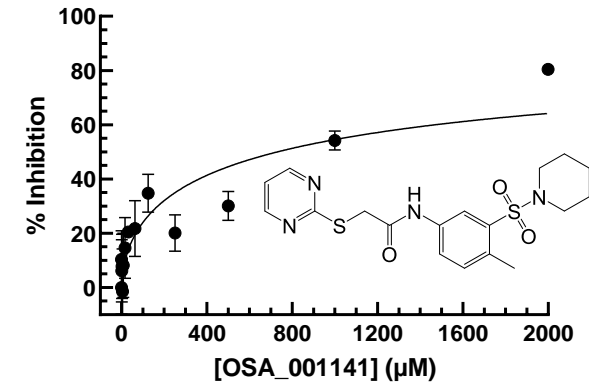
OSA_001169

IC50 = 25.37 ± 3.74 μM



OSA_001141

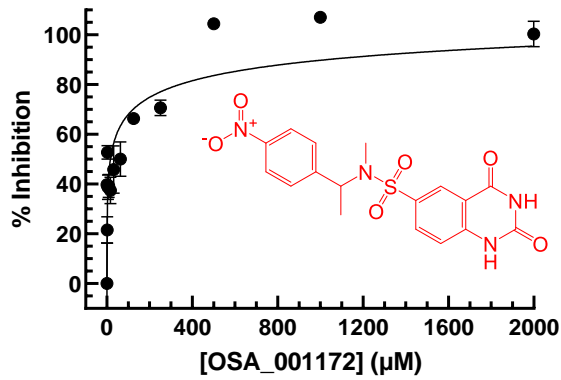
IC50 = 729.9 ± 171.8 μM



Characterization of inhibition of *P. aeruginosa* MurC by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Dose Response Curves (3)

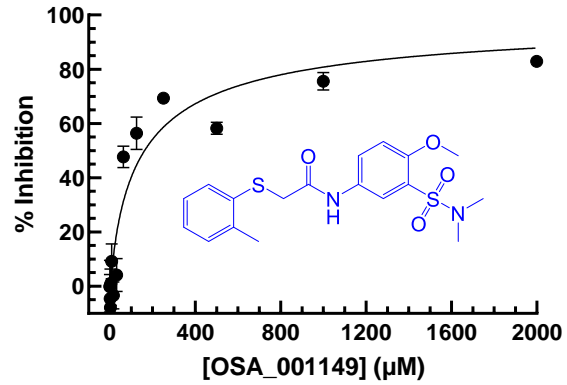
OSA_001172

IC50 = 73.82 ± 182.9 μM



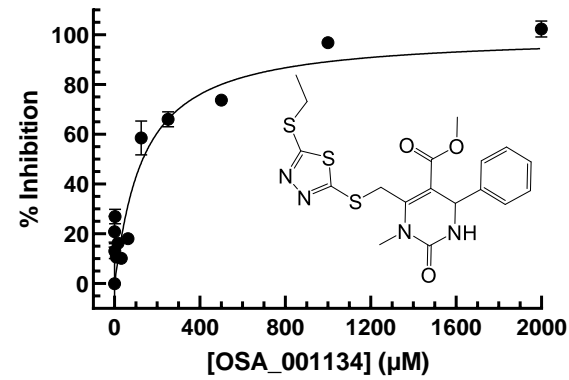
OSA_001149

IC50 = 140.1 ± 27.54 μM



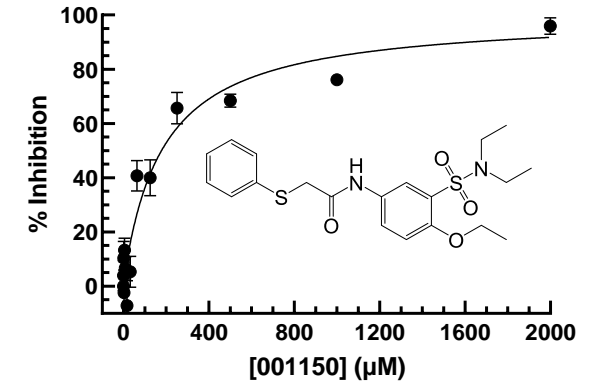
OSA_001134

IC50 = 132.0 ± 23.54 μM



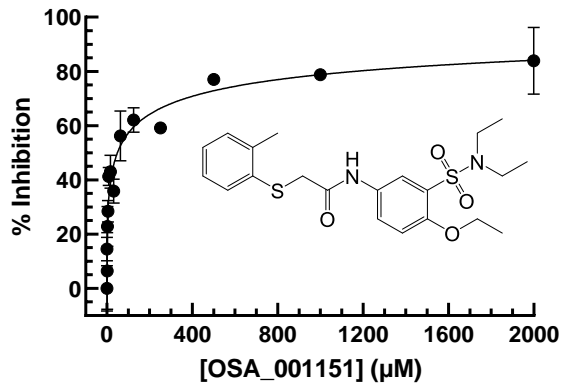
OSA_001150

IC50 = 184.0 ± 25.6 μM



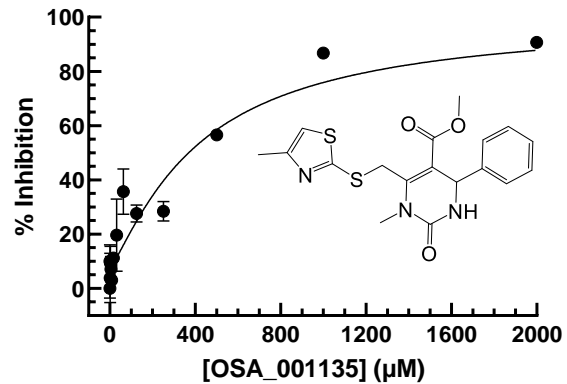
OSA_001151

IC50 = 80.00 ± 93.02 μM



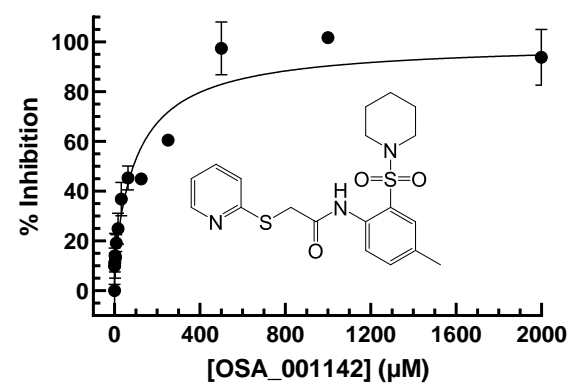
OSA_001135

IC50 = 422.3 ± 157.2 μM



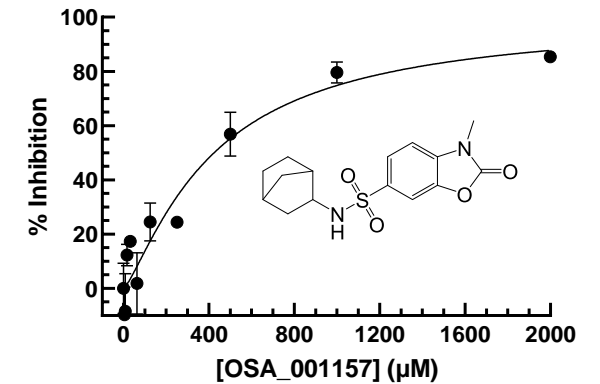
OSA_001142

IC50 = 109.8 ± 17.5 μM



OSA_001157

IC50 = 423.9 ± 66.02 μM



Inhibition of *P. aeruginosa* MurC by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: IC50 relationships within structurally similar inhibitors

IC50 (μM) \pm SD (n = 3)

Acyl thio-N-Phenyl acetamides

22.1 \pm 2.69

33.8 \pm 4.38

80.00 \pm 93.02

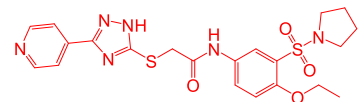
109.8 \pm 17.5

130.9 \pm 30.2

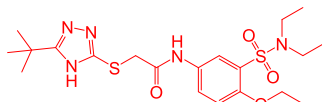
140.1 \pm 27.54

184.0 \pm 25.6

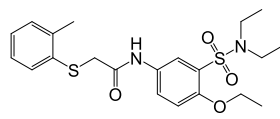
729.9 \pm 171.8



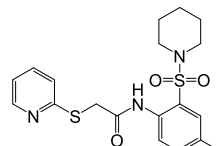
OSA_001147



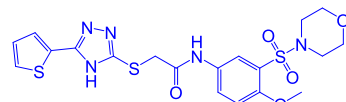
OSA_001145



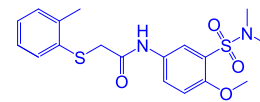
OSA_001151



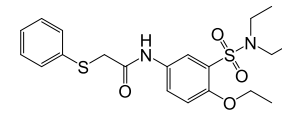
OSA_001142



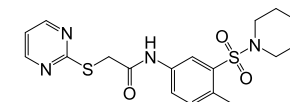
OSA_001148



OSA_001149



OSA_001150



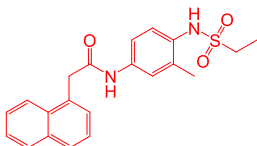
OSA_001141

Naphthyl phenyl sulfonamides

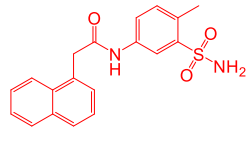
58.00 \pm 6.20

244.5 \pm 41.3

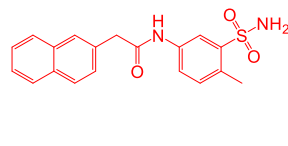
299.1 \pm 95.07



OSA_001155



OSA_001156



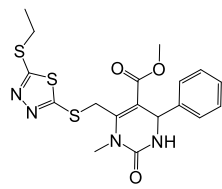
OSA_001153

Thiadiazol phenyl pyrimidines

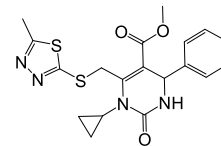
132.0 \pm 23.54

238.6 \pm 114.7

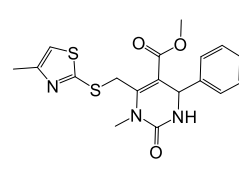
422.3 \pm 157.2



OSA_001134



OSA_001136

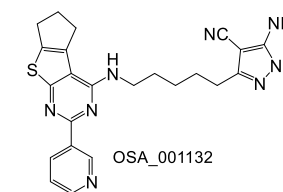


OSA_001135

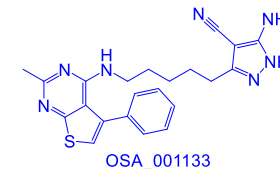
Amino pentyl pyrazoles

14.6 \pm 1.33

25.5 \pm 3.15



OSA_001132



OSA_001133

Tetrahydroquinazoline-6-sulfonamides

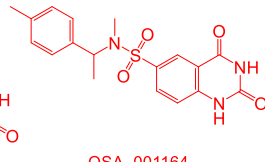
73.82 \pm 182.9

124.3 \pm 19.2

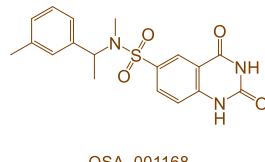
127.4 \pm 17.2



OSA_001172



OSA_001164



OSA_001168

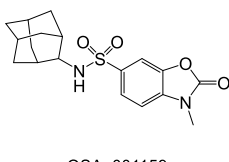
Acyl benzoxazole sulfonamides

19.6 \pm 18.6

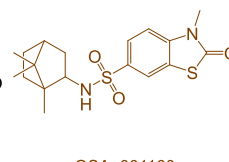
38.0 \pm 13.1

68.2 \pm 15.2

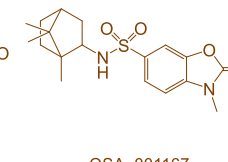
423.9 \pm 66.02



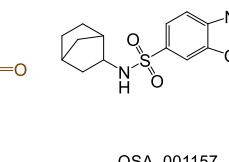
OSA_001159



OSA_001160



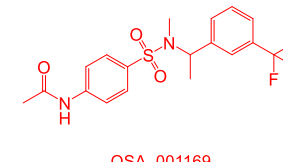
OSA_001167



OSA_001157

Sulfamoyl phenyl acetamides

25.37 \pm 3.74

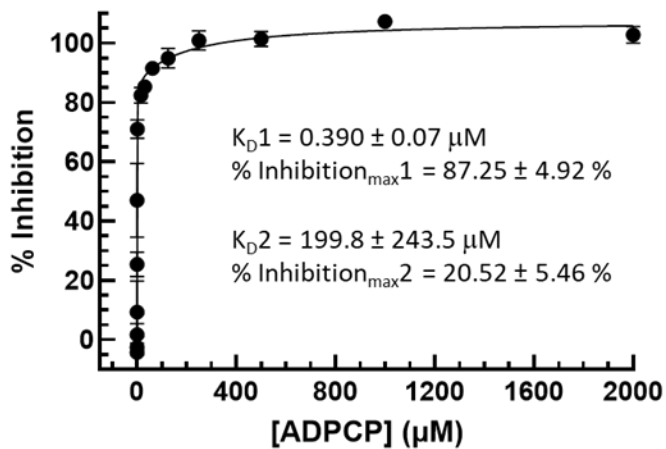


OSA_001169

Characterization of interaction of *P. aeruginosa* MurC with ATP substrate or ADPCP (ATP analogue) inhibitor

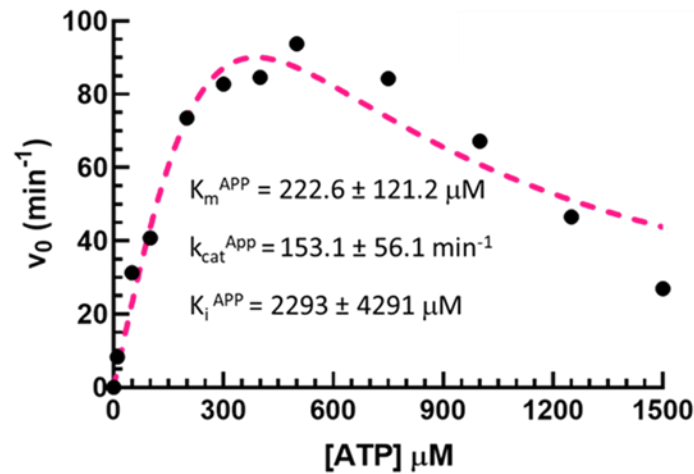
Inhibition of MurC by ADPCP

Biphasic two K_D values



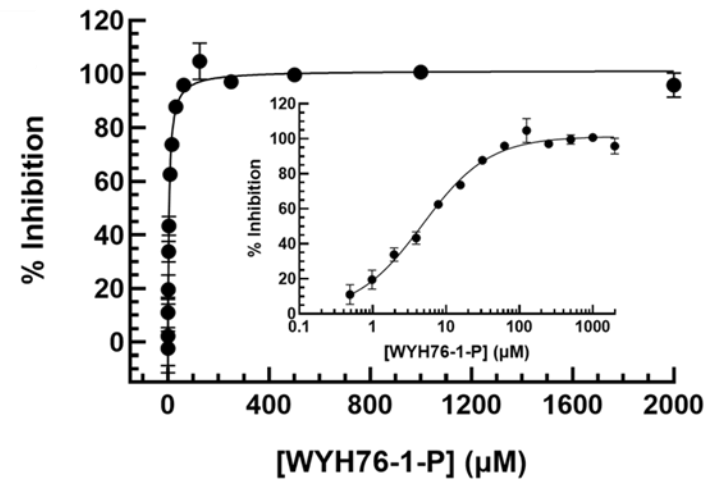
Dependence of MurC velocity on ATP concentration

Substrate Inhibition

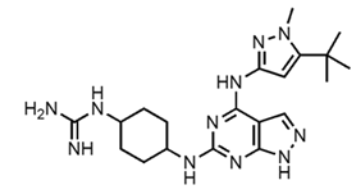


Inhibition of MurC by WYH76-1-P

Monophasic one K_D value



WYH76-1-P



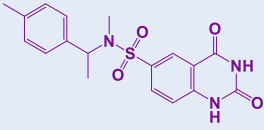
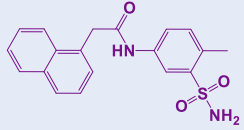
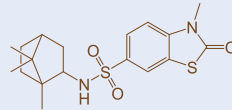
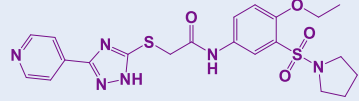
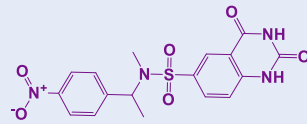
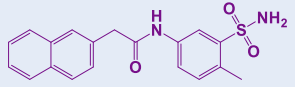
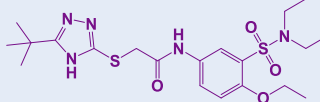
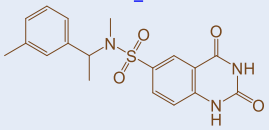
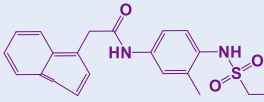
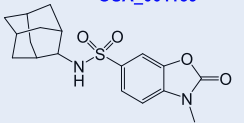
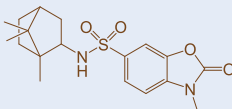
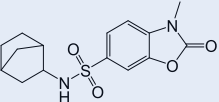
Data suggest that ADPCP mimics ATP in that it binds in two different ways to MurC, in contrast to the behaviour of the pyrazolopyrimidines

OSA Compounds at 0.5 mM sent by Yiwei Wang on the 23rd May and 25th July, 2023 found to exert >50% inhibition of *P. aeruginosa* MurF activity, colour coded relative to multiple Mur ligase targeting.

MurF Assay:

Assays were performed at 30°C in a 10 µl volume/384 well format, containing 50 mM MOPS, 10 mM MgCl₂, 0.5 mM inosine, 2.5 mM.min⁻¹ *Arthrobacter* sp. xanthine oxidase, 20 mM.min⁻¹ horse radish peroxidase, 50 µM amplex Red, 2.64 mM.min⁻¹ *E. coli* purine nucleoside phosphorylase, 20.1 µM ATP, 0.1 mM UDP-MurNAc-L-Ala-γ-D-Glu-meso-Diaminopimelate, 55.8 nM *P. aeruginosa* MurF and where added, 1 mM D-alanyl-D-alanine. OSA compounds were added from 50 mM stocks in DMSO, where the final concentrations of DMSO and compound were 1 % (v/v) and 0.5 mM respectively. Controls without compound contained 1% (v/v) DMSO. ADPCP if added was at 0.4 mM. Per compound/DMSO/ADPCP, MurF was assayed in three wells where reaction was initiated by addition of D-alanyl-D-alanine and in three wells where reaction (control) was initiated by water. The fluorescent product of the reaction cascade (resorufin – derived from amplex red) was continuously monitored from above the well at an excitation and emission wavelength of 545 nm and 585 nm respectively in a Varioskan Lux plate reader.

Compounds with > 50% Potency vs *P. aeruginosa* MurF

	% Inhibition (mean ± SD)		% Inhibition (mean ± SD)
	95.8 ± 2.2		67.3 ± 8.6
	77.4 ± 8.7		67.0 ± 4.0
	72.1 ± 3.6		61.9 ± 2.1
	71.7 ± 2.6		59.9 ± 7.3
	69.4 ± 5.5		56.7 ± 1.7
	69.1 ± 7.3		51.8 ± 3.9

Maroon coloured compounds are >50% inhibitors of MurC, D, E and F, brown compounds are >50% inhibitors of MurC, D and F, black compounds are >50% inhibitors of MurC and MurF.

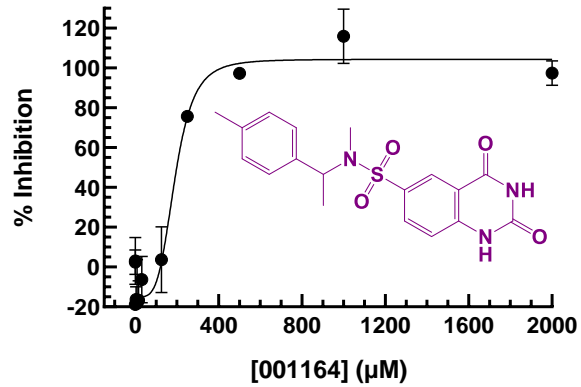
Characterization of inhibition of *P. aeruginosa* MurF by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Complete Data Set

Compound	IC50 ($\mu\text{M} \pm \text{SD}$) (n=3)	Hill Coefficient	Original Hit % Inhibition at 0.5 mM
ADPCP	1.459 \pm 0.32	0.78 \pm 0.32	91.5 \pm 1.7
OSA_001164	190.4 \pm 16.36	4.21 \pm 0.97	95.8 \pm 2.2
OSA_001160	261.0 \pm 32.34	1.80 \pm 0.33	77.4 \pm 8.7
OSA_001172	343.9 \pm 36.82	4.31 \pm 1.25	72.1 \pm 3.6
OSA_001145	93.05 \pm 7.01	4.76 \pm 1.04	71.7 \pm 2.6
OSA_001155	85.54 \pm 18.24	0.49 \pm 0.05	69.4 \pm 5.5
OSA_001167	99.41 \pm 19.04	1.49 \pm 0.36	69.1 \pm 7.3
OSA_001156	639.2 \pm 78.70	1.85 \pm 0.42	67.3 \pm 8.6
OSA_001147	339.8 \pm 87.50	1.59 \pm 0.49	67.0 \pm 4.0
OSA_001153	668.3 \pm 46.17	2.83 \pm 0.16	61.9 \pm 2.1
OSA_001168	216.8 \pm 71.18	1.09 \pm 0.25	59.9 \pm 7.3
OSA_001159	384.3 \pm 29.93	1.75 \pm 0.22	56.7 \pm 1.7
OSA_001157	1917 \pm 176.9	1.60 \pm 0.27	51.8 \pm 3.9

Characterization of inhibition of *P. aeruginosa* MurF by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Dose Response Curves (1)

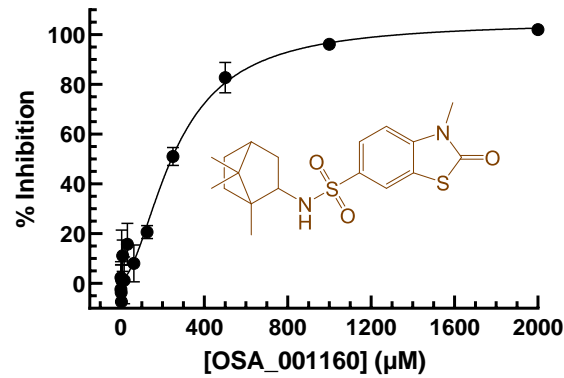
OSA_001164

IC50 = 190.4 ± 16.36 μM



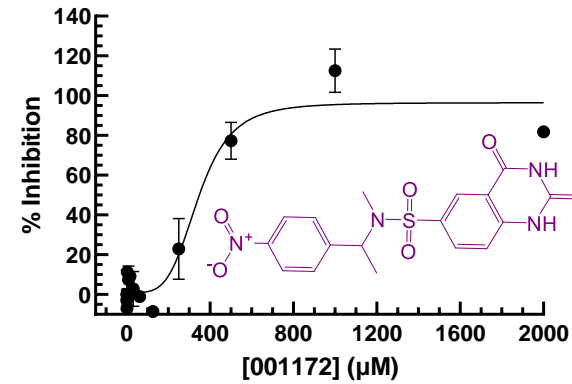
OSA_001160

IC50 = 261.0 ± 32.34 μM



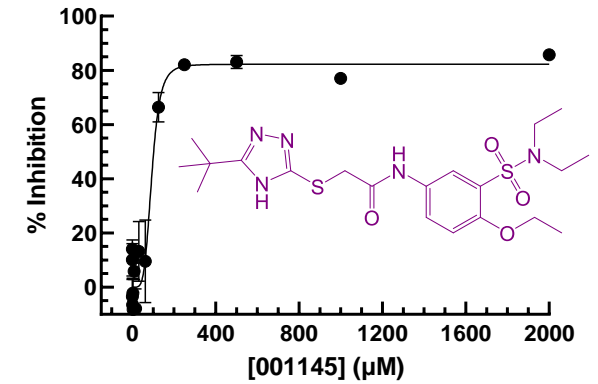
OSA_001172

IC50 = 343.9 ± 36.82 μM



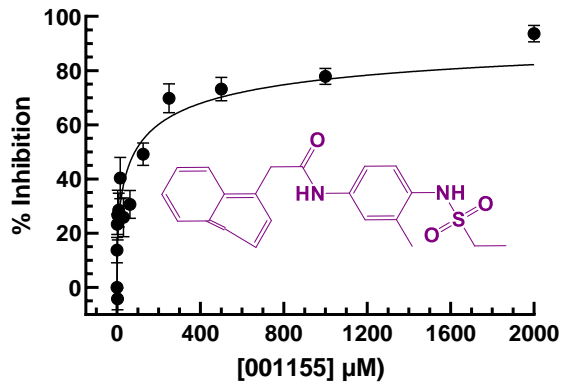
OSA_001145

IC50 = 93.05 ± 7.01 μM



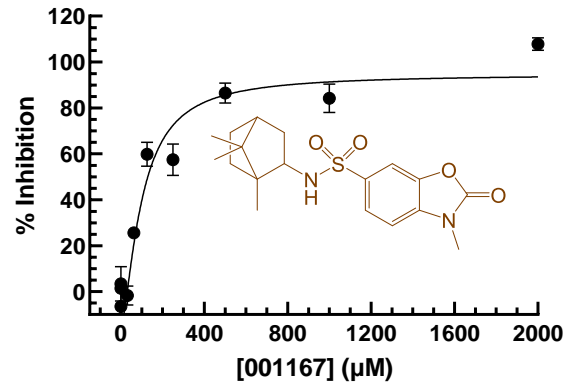
OSA_001155

IC50 = 85.54 ± 18.24 μM



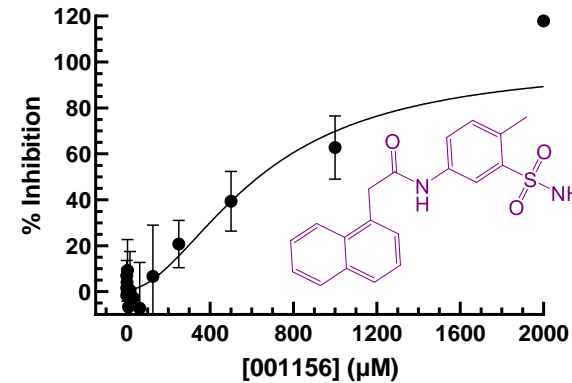
OSA_001167

IC50 = 99.41 ± 19.04 μM



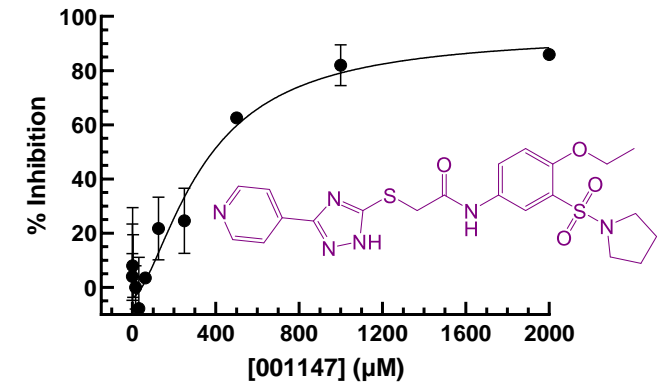
OSA_001156

IC50 = 639.2 ± 78.70 μM



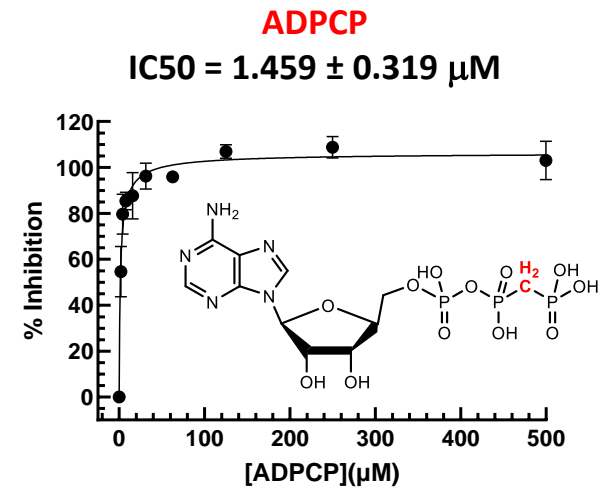
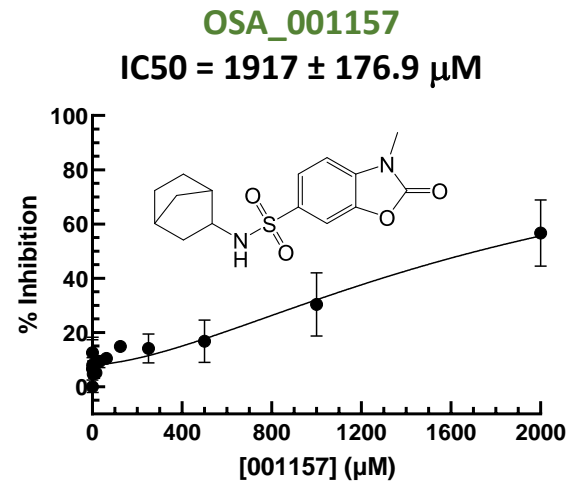
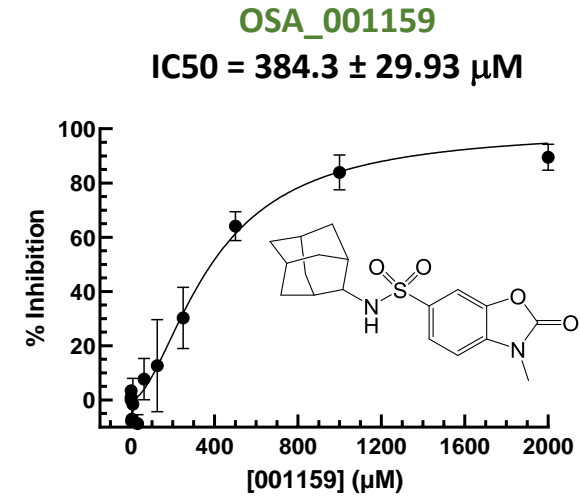
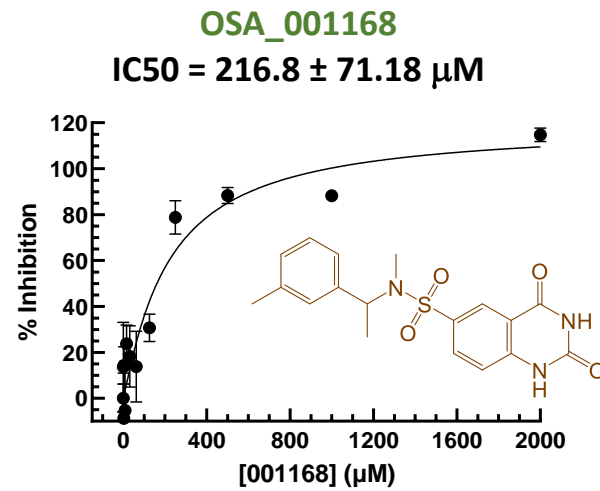
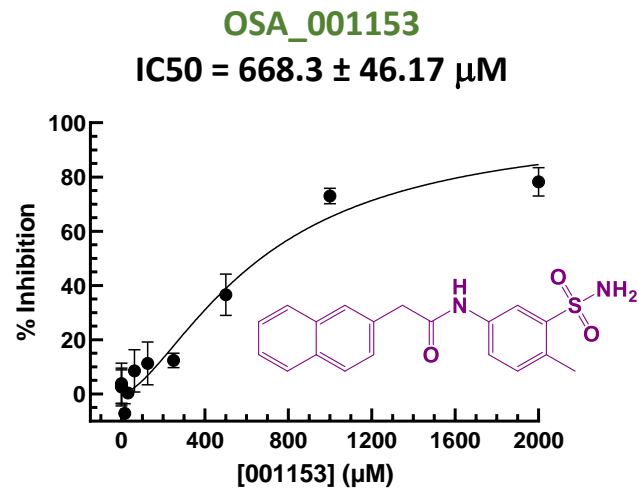
OSA_001147

IC50 = 339.8 ± 87.50 μM



Maroon coloured compounds are >50% inhibitors of MurC, D, E and F, brown compounds are >50% inhibitors of MurC, D and F, black compounds are >50% inhibitors of MurC and MurF.

Characterization of inhibition of *P. aeruginosa* MurF by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Dose Response Curves (2)



Maroon coloured compounds are >50% inhibitors of MurC, D, E and F, brown compounds are >50% inhibitors of MurC, D and F, black compounds are >50% inhibitors of MurC and MurF.

Collated IC50s of *P. aeruginosa* MurC, D, E and F vs all OSA Compounds exerting more than 50 % inhibition at 0.5 mM sent by Yiwei Wang on the 23rd May and 25th July, 2023

Compound	IC50 vs <i>Pseudomonas aeruginosa</i> Mur Ligase							
	MurC		MurD		MurE		MurF	
	IC50 (μM)	Hill Coefficient	IC50 (μM)	Hill Coefficient	IC50 (μM)	Hill Coefficient	IC50 (μM)	Hill Coefficient
ADPCP	0.38 ± 0.04	1.50 ± 0.23	49.03 ± 4.88	1.10 ± 0.11	0.35 ± 0.08	0.68 ± 0.09	1.46 ± 0.32	0.78 ± 0.32
OSA_001147	22.1 ± 2.69	2.67 ± 0.73	548.1 ± 132	1.15 ± 0.31	588.4 ± 117	0.53 ± 0.07	339.8 ± 87.50	1.59 ± 0.49
OSA_001145	38.8 ± 4.38	1.81 ± 0.37	615.3 ± 200	0.35 ± 0.06	36.6 ± 8.76	0.46 ± 0.05	93.05 ± 7.01	4.76 ± 1.04
OSA_001133	25.5 ± 3.15	2.52 ± 0.67			5093 ± 3091	0.27 ± 0.06		
OSA_001132	14.6 ± 1.33	1.73 ± 0.24						
OSA_001167	68.2 ± 15.2	1.24 ± 0.31	538.0 ± 109	0.95 ± 0.19			99.41 ± 19.04	1.49 ± 0.36
OSA_001159	19.6 ± 18.6	0.45 ± 0.14					384.3 ± 29.93	1.75 ± 0.22
OSA_001160	38.0 ± 13.1	0.67 ± 0.13	253.0 ± 45.0	0.67 ± 0.08			261.0 ± 32.34	1.80 ± 0.33
OSA_001164	124.3 ± 19.2	2.63 ± 0.96	232.2 ± 40.1	0.81 ± 0.11	442.1 ± 80.0	1.73 ± 0.38	190.4 ± 16.36	4.21 ± 0.97
OSA_001148	130.9 ± 30.2	1.04 ± 0.20			392.2 ± 67.1	0.57 ± 0.07		
OSA_001156	224.5 ± 41.3	1.21 ± 0.22	378.5 ± 58.0	1.12 ± 0.18	2667 ± 945	0.39 ± 0.08	639.2 ± 78.70	1.85 ± 0.42
OSA_001155	58.0 ± 6.20	1.74 ± 0.29	713.1 ± 132	1.16 ± 0.25	267.3 ± 47.3	0.68 ± 0.09	85.54 ± 18.24	0.49 ± 0.05
OSA_001168	127.4 ± 17.2	1.82 ± 0.39	441.4 ± 51.4	1.74 ± 0.31			216.8 ± 71.18	1.09 ± 0.25
OSA_001136	238.6 ± 114.7	0.62 ± 0.16						
OSA_001153	299.1 ± 95.07	1.00 ± 0.18	722.1 ± 135	1.12 ± 0.25	950.6 ± 150	0.82 ± 0.13	668.3 ± 46.17	2.83 ± 0.16
OSA_001169	25.37 ± 3.74	1.48 ± 0.29	24581 ± 22981	0.41 ± 0.12	56.32 ± 23.39	0.25 ± 0.026		
OSA_001141	729.9 ± 171.8	0.58 ± 0.08						
OSA_001172	73.82 ± 182.9	0.31 ± 0.11	426.2 ± 188	1.12 ± 0.32	679.3 ± 64.0	2.66 ± 0.55	343.9 ± 36.82	4.31 ± 1.25
OSA_001149	140.1 ± 27.54	0.77 ± 0.10			351.9 ± 94.1	0.48 ± 0.08		
OSA_001134	132.0 ± 23.54	1.04 ± 0.19						
OSA_001150	184.0 ± 25.6	1.00 ± 0.12						
OSA_001151	80.00 ± 93.02	0.38 ± 0.10						
OSA_001135	422.3 ± 157.2	1.21 ± 0.33						
OSA_001142	109.8 ± 17.5	0.96 ± 0.13						
OSA_001157	423.9 ± 66.02	1.27 ± 0.24					1917 ± 176.9	1.60 ± 0.27

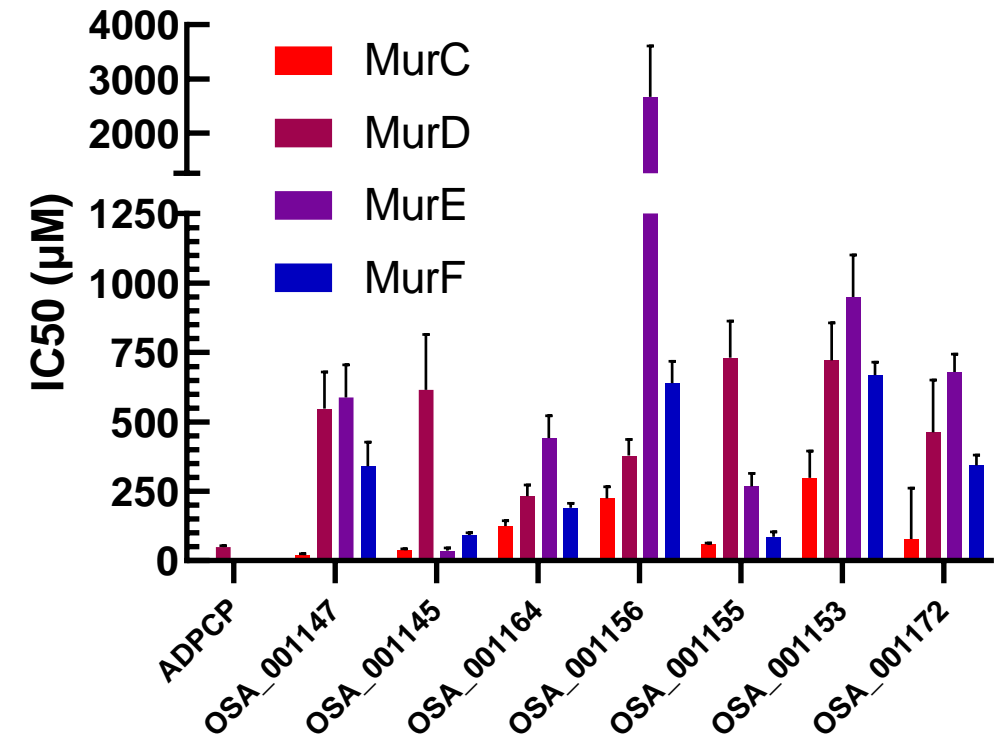
All data are mean ± SD for triplicate data. Gaps indicate that that particular inhibitor/ligase combination failed to register greater than 50 % inhibition at 0.5 mM OSA compound in the initial screen.

Comparison of potency of 0.5 mM OSA Compounds sent by Yiwei Wang on the 23rd May and 25th July, 2023 targeting *P. aeruginosa* MurC, D, E and F vs. their corresponding IC50 values

Previous single shot 0.5 mM compound inhibition data

Compound (0.5 mM)	% Inhibition (mean \pm SD (n = 3));			
	<i>P. aeruginosa</i> MurC	<i>P. aeruginosa</i> MurD	<i>P. aeruginosa</i> MurE	<i>P. aeruginosa</i> MurF
OSA_001147	98.7 \pm 1.0	60.4 \pm 2.6	55.9 \pm 6.3	67.0 \pm 4.0
OSA_001145	97.4 \pm 1.5	108.9 \pm 15.1	67.3 \pm 0.8	71.7 \pm 2.6
OSA_001164	91.1 \pm 1.3	89.4 \pm 9.8	76.0 \pm 9.0	95.8 \pm 2.2
OSA_001156	86.4 \pm 2.2	69.6 \pm 9.5	56.1 \pm 5.8	67.3 \pm 8.6
OSA_001155	86.2 \pm 2.6	87.9 \pm 4.3	69.3 \pm 4.0	69.4 \pm 5.5
OSA_001153	83.7 \pm 3.5	75.0 \pm 3.1	55.0 \pm 5.6	61.9 \pm 2.0
OSA_001172	81.5 \pm 1.7	72.7 \pm 6.2	78.0 \pm 8.4	72.1 \pm 3.6

Corresponding IC50 determinations

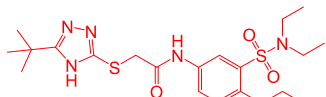


- Clearly, for all OSA compounds, exerting >50% inhibition in the initial screen at 0.5 mM, MurC is more susceptible to inhibition than MurD, E and F as determined by IC50.
- Of the seven OSA compounds that targeted all ligases, the IC50 values were clearly lowest for MurC (top right).
- Single, (MurC) Dual (MurC and E, MurC and F) or triple targeting (MurC, E and F) with IC50 values below 100 µM were observed

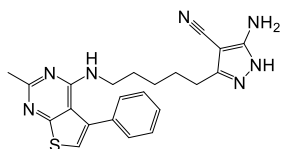
Single, doubly and triply targeting OSA Compounds sent by Yiwei Wang on the 23rd May and 25th July, 2023 targeting *P. aeruginosa* MurC, E and F as triaged at a maximum IC50 of 100 μ M

Notably, MurD is not targeted by any OSA compound with an IC50 below 100 μ M

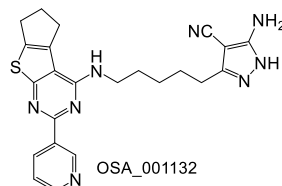
Compound	Sub 100 μ M IC50 vs <i>Pseudomonas aeruginosa</i> Mur Ligase								Targeting
	MurC		MurD		MurE		MurF		
	IC50 (μ M)	Hill Coefficient	IC50 (μ M)	Hill Coefficient	IC50 (μ M)	Hill Coefficient	IC50 (μ M)	Hill Coefficient	
OSA_001145	38.8 \pm 4.38	1.81 \pm 0.37	← IC50 \geq 100 μ M →		36.6 \pm 8.76	0.46 \pm 0.05	93.05 \pm 7.01	4.76 \pm 1.04	Triple (C,E,F)
OSA_001133	25.5 \pm 3.15	2.52 \pm 0.67	← IC50 \geq 100 μ M →						Single
OSA_001132	14.6 \pm 1.33	1.73 \pm 0.24	← IC50 \geq 100 μ M →						Single
OSA_001159	19.6 \pm 18.6	0.45 \pm 0.14	← IC50 \geq 100 μ M →						Single
OSA_001160	38.0 \pm 13.1	0.67 \pm 0.13	← IC50 \geq 100 μ M →						Single
OSA_001155	58.0 \pm 6.20	1.74 \pm 0.29	← IC50 \geq 100 μ M →				85.54 \pm 18.24	0.49 \pm 0.05	Double (C,F)
OSA_001169	25.37 \pm 3.74	1.48 \pm 0.29	← IC50 \geq 100 μ M →		56.32 \pm 23.39	0.25 \pm 0.026			Double (C,E)
OSA_001151	80.00 \pm 93.02	0.38 \pm 0.10	← IC50 \geq 100 μ M →						Single



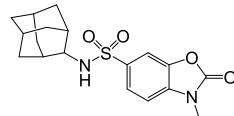
OSA_001145



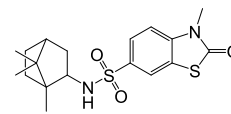
OSA_001133



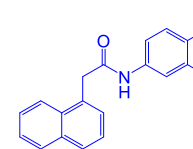
OSA_001132



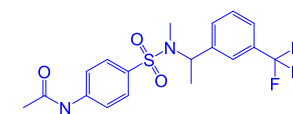
OSA_001159



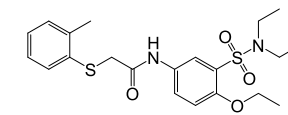
OSA_001160



OSA_001155



OSA_001169



OSA_001151