Evaluation of pyrazolopyrimidines as MurC inhibitors received in 2024 (Yuhang)
and
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

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14th May, 2024

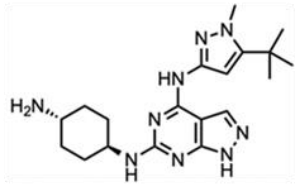
Further evaluation of pyrazolopyrimidines as *P. aeruginosa* MurC inhibitors (Yuhang)

MurC Assay:

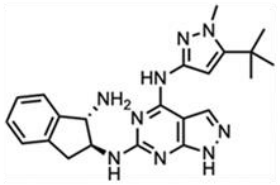
Assays were performed at 30°C in a 10 µl volume/384 well format, containing 50 mM MOPS, 10 mM $MgCl_2$, 0.5 mM inosine, 2.5 $mM.min^{-1}$ *Arthrobacter* sp. xanthine oxidase, 20 $mM.min^{-1}$ horse radish peroxidase, 50 µM amplex Red, 2.64 $mM.min^{-1}$ *E. coli* purine nucleoside phosphorylase, 60 µM ATP (where added), 0.1 mM UDP-MurNAc, 15.1 nM *P. aeruginosa* MurC and 5mM L-alanine. WYH compounds were added from 43, 48 or 49 mM stocks in DMSO, where the final concentrations of DMSO and compound were 1.16 % (v/v) and in the range 0.49 to 1600, 1920 or 2000 µM as indicated. Controls without compound contained 1.16% (v/v) DMSO. Per compound 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 IC_{50} values and Hill coefficients. Data were further fitted to the Morrison equation for tight binding inhibitors assuming ATP-competitive inhibition (GraphPad 9.1 standard equation menu), to extract estimates of compound K_i.

Characterization of *P. aeruginosa* MurC inhibition by Yuhang's free amine and guanidinium derivative pyrazolopyrimidines received 24th April, 2024

WYH30-X-P



WYH85-2-P



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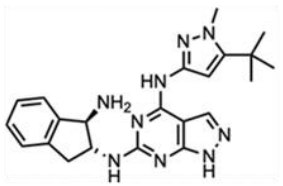

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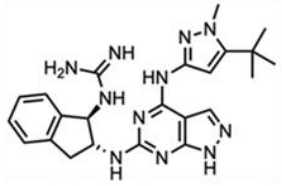
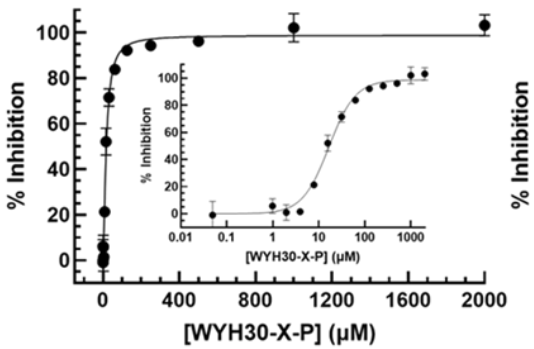
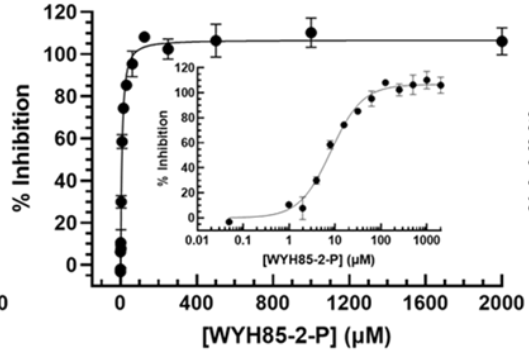


Impact of Yuhang's Pyrazolopyrimidines received 24-04-24 on *P. aeruginosa* MurC: IC50 Determinations

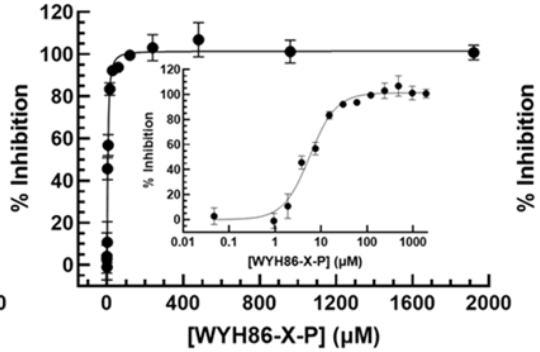
IC_{50} = 16.58 ± 0.96 µM
Hill Coeff. = 1.51 ±0.12



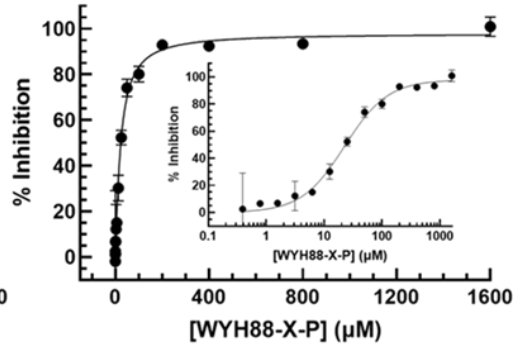
IC_{50} = 8.11 ± 0.60 µM
Hill Coeff. = 1.26 ± 0.11



IC_{50} = 5.66 ± 0.41 µM
Hill Coeff. = 1.51 ±0.15

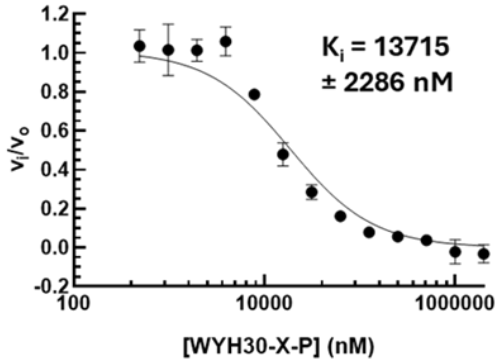


IC_{50} = 22.59 ± 2.14 µM
Hill Coeff. = 1.17 ±0.11

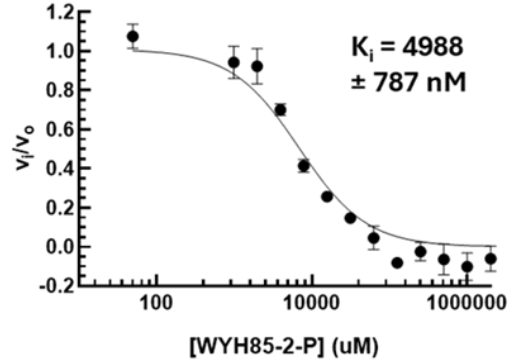


Impact of Yuhang's Pyrazolopyrimidines received 24-04-24 on *P. aeruginosa* MurC: Ki Determinations (Morrison)

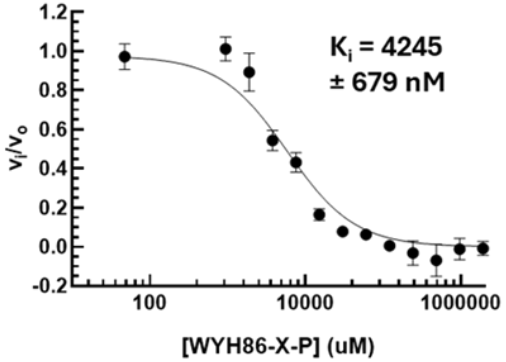
K_i = 13715 ± 2286 nM



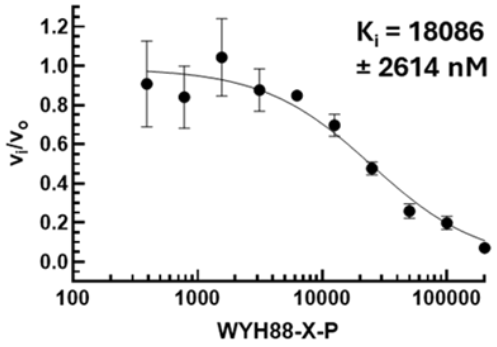
K_i = 4988 ± 787 nM



K_i = 4245 ± 679 nM

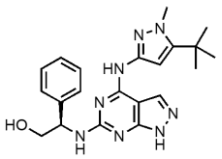


K_i = 18086 ± 2614 nM

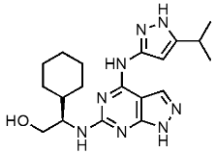


Characterization of *P. aeruginosa* MurC inhibition by Yuhang's free amine and guanidinium derivative pyrazolopyrimidines received 24th April, 2024 and previous derivatives sent in February 2024

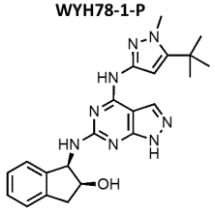
WYH25-5-P



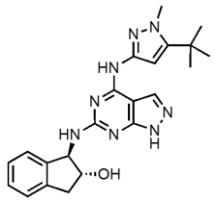
WYH9-4-P



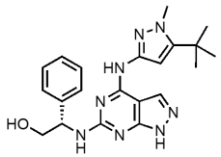
WYH78-1-P



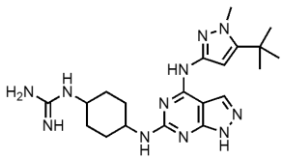
WYH77-1-P



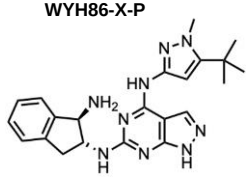
WYH17-15-P



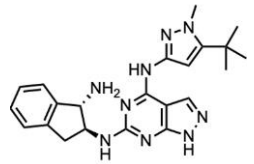
WYH76-1-P



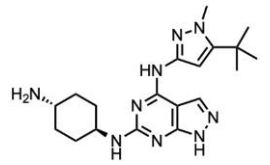
WYH86-X-P



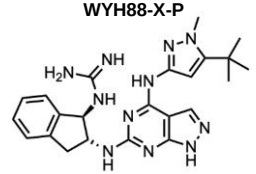
WYH85-2-P



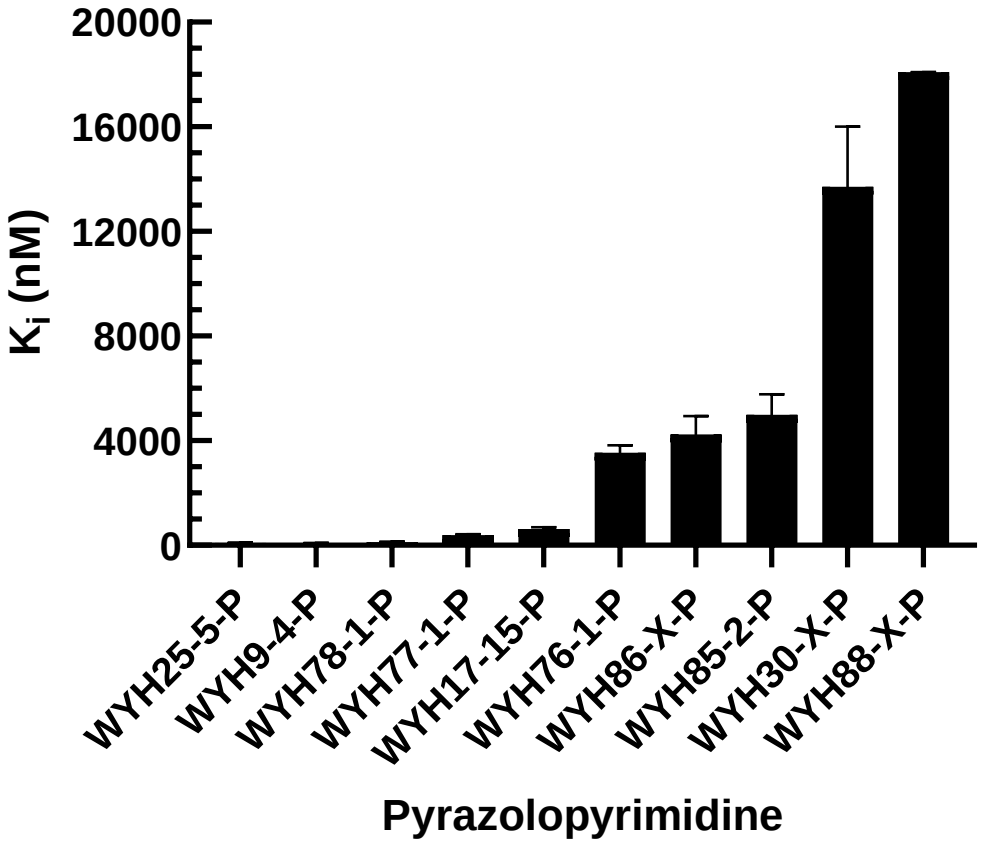
WYH30-X-P



WYH88-X-P



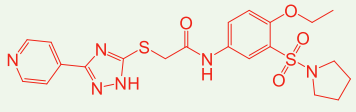
Compound	Received	IC_{50} (μM) ± SD (n = 3)	Hill Coeff. ± SD (n = 3)	K_i (nM) ± SD (n = 3)
WYH25-5-P	February	0.115 ± 0.022	0.68 ± 0.08	89.8 ± 13.1
WYH9-4-P	February	0.143 ± 0.024	1.03 ± 0.15	74.5 ± 15.2
WYH78-1-P	February	0.200 ± 0.022	1.38 ± 0.17	123 ± 15.4
WYH77-1-P	February	0.663 ± 0.057	1.21 ± 0.12	391 ± 32.4
WYH17-15-P	February	1.061 ± 0.197	0.77 ± 0.10	618 ± 78.9
WYH76-1-P	February	4.778 ± 0.463	0.96 ± 0.08	3540 ± 287
WYH86-X-P	April	5.660 ± 0.410	1.51 ± 0.15	4245 ± 697
WYH85-2-P	April	8.110 ± 0.600	1.26 ± 0.11	4988 ± 787
WYH30-X-P	April	16.58 ± 0.960	1.51 ± 0.12	13715 ± 2286
WYH88-X-P	April	22.59 ± 2.140	1.17 ± 0.11	18086 ± 13.1



Ki calculated assuming competitive inhibition with respect to ATP

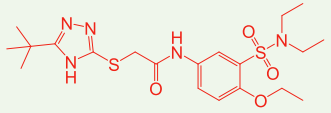
OSA Compounds at 0.5 mM sent by Yiwei Wang on the 23rd May and 25th July, 2023 found to exert >50% inhibition of *P. aeruginosa* MurC activity, colour coded relative to multiple Mur ligase targeting.

98.66 ± 1.02



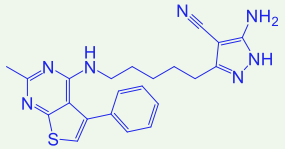
OSA_001147

97.38 ± 1.47



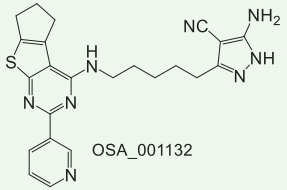
OSA_001145

96.29 ± 2.04



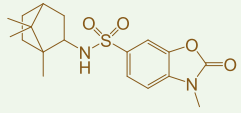
OSA_001133

96.12 ± 1.50



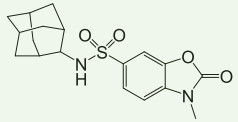
OSA_001132

92.73 ± 2.56



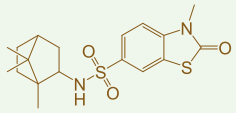
OSA_001167

92.26 ± 10.00



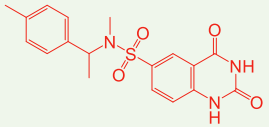
OSA_001159

91.91 ± 7.38



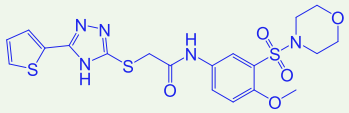
OSA_001160

91.12 ± 1.33



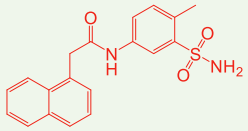
OSA_001164

88.08 ± 1.17



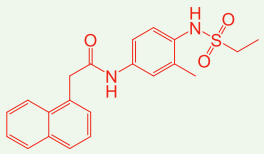
OSA_001148

86.36 ± 2.21



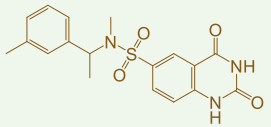
OSA_001156

86.16 ± 2.56



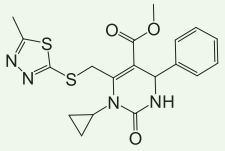
OSA_001155

84.76 ± 4.99



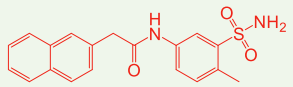
OSA_001168

83.75 ± 2.04



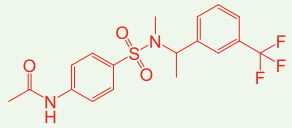
OSA_001136

83.66 ± 3.47



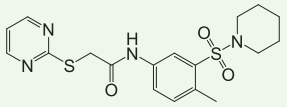
OSA_001153

82.82 ± 2.54



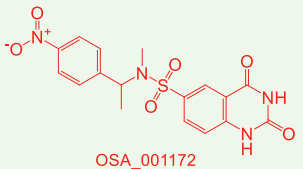
OSA_001169

82.41 ± 0.98



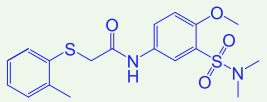
OSA_001141

81.51 ± 1.68



OSA_001172

76.69 ± 2.75

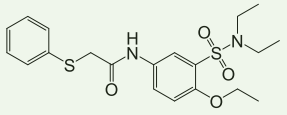


OSA_001149

75.71 ± 6.33

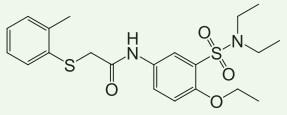
OSA_001134

68.73 ± 5.65



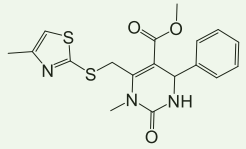
OSA_001150

63.28 ± 8.09



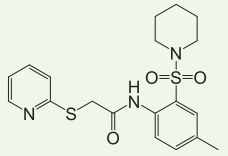
OSA_001151

63.24 ± 4.05



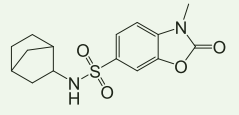
OSA_001135

61.81 ± 3.61



OSA_001142

53.55 ± 7.96



OSA_001157

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

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.

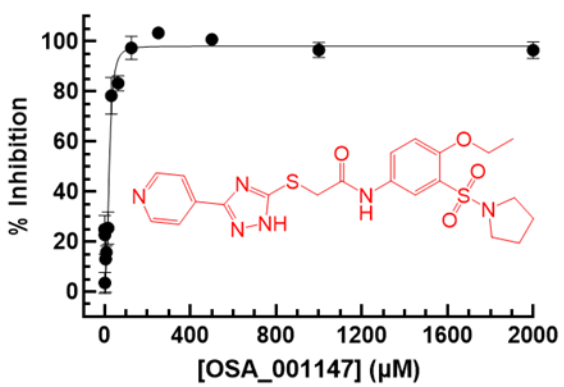
Characterization of inhibition of *P. aeruginosa* MurC by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Complete Data Set

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

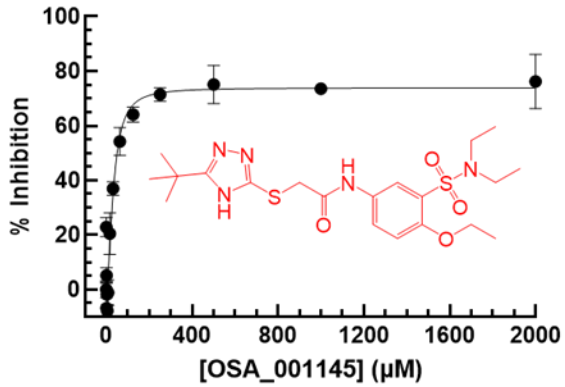
OSA_001147

IC_{50} = 22.1 ± 2.69 µM



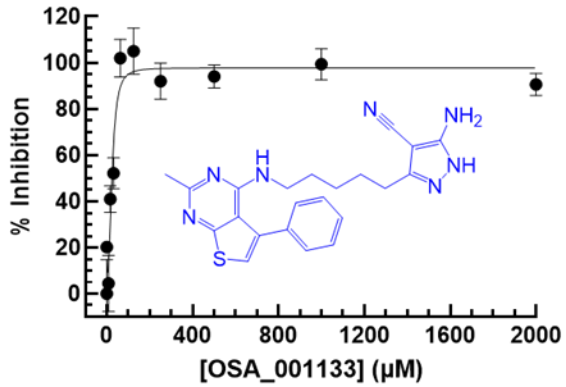
OSA_001145

IC_{50} = 33.8 ± 4.38 µM



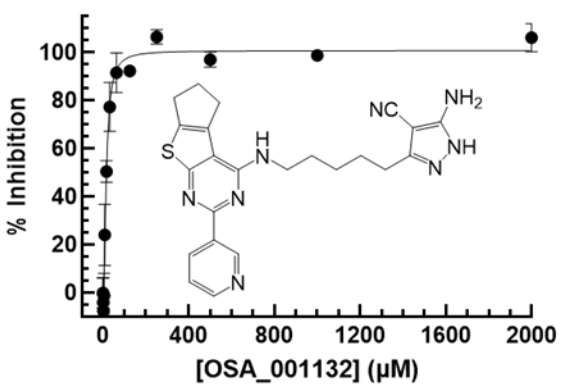
OSA_001133

IC_{50} = 25.5 ± 3.15 µM



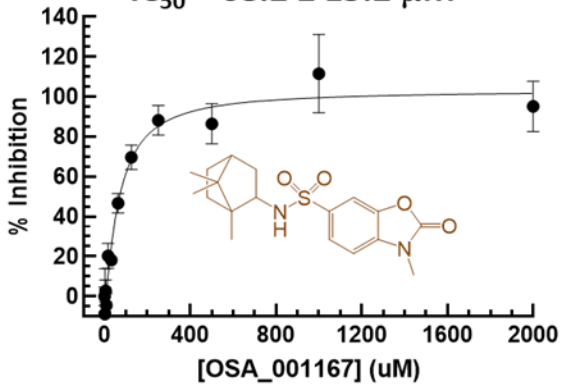
OSA_001132

IC_{50} = 14.6 ± 1.33 µM



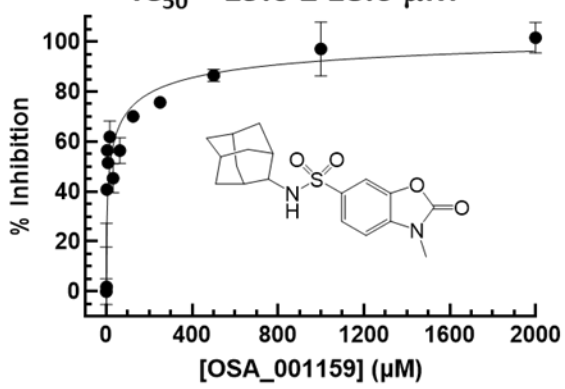
OSA_001167

IC_{50} = 68.2 ± 15.2 µM



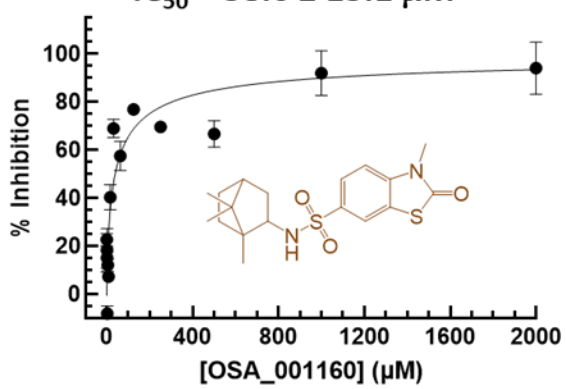
OSA_001159

IC_{50} = 19.6 ± 18.6 µM



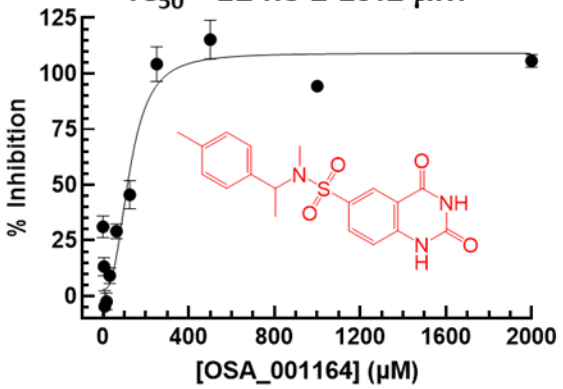
OSA_001160

IC_{50} = 38.0 ± 13.1 µM



OSA_001164

IC_{50} = 124.3 ± 19.2 µM

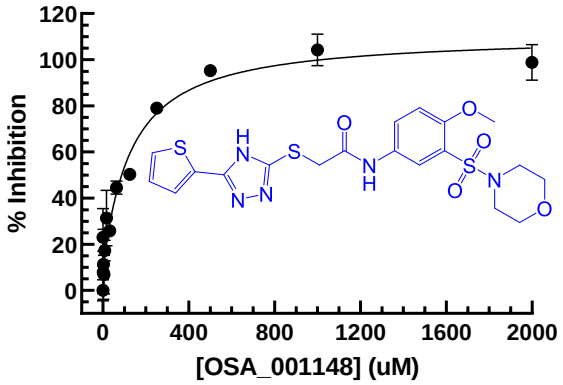


Red structures: MurC inhibitors with >50% potency vs MurD and E; Blue structures: MurC inhibitors with > 50% potency vs MurE;
Brown structures: MurC inhibitors with >50% potency vs MurD.

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)

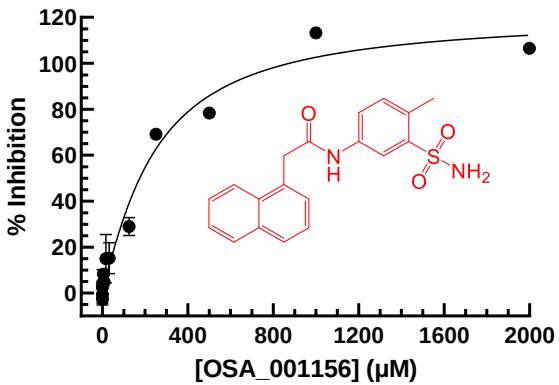
OSA_001148

IC50 = 130.9 ± 30.2 µM



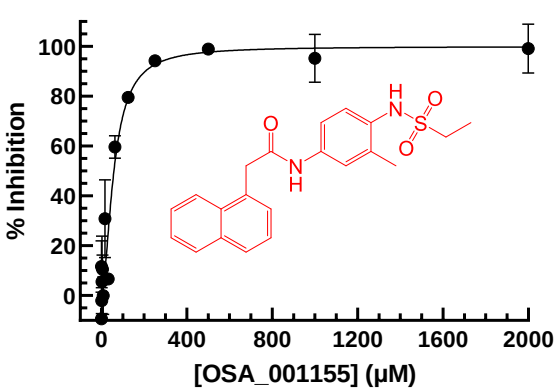
OSA_001156

IC50 = 244.5 ± 41.3 µM



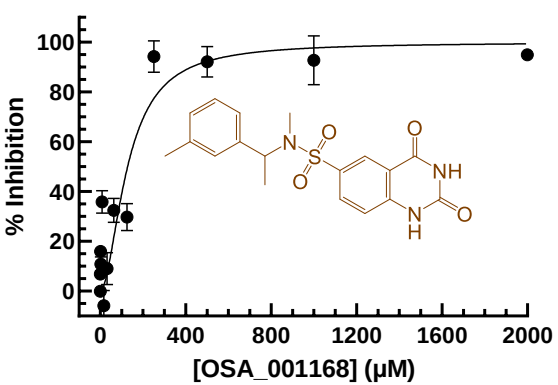
OSA_001155

IC50 = 58.00 ± 6.20 µM



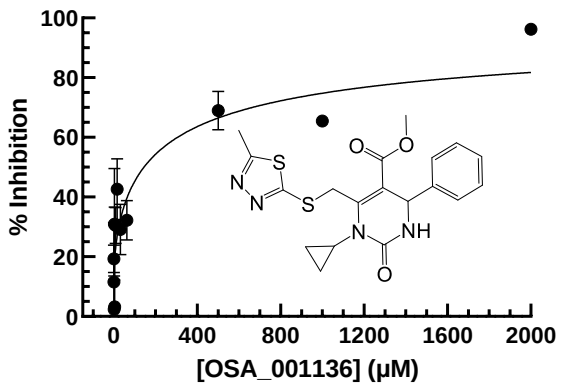
OSA_001168

IC50 = 127.4 ± 17.2 µM



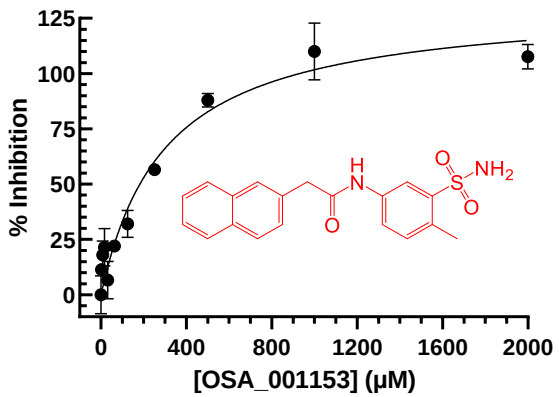
OSA_001136

IC50 = 238.6 ± 114.7 µM



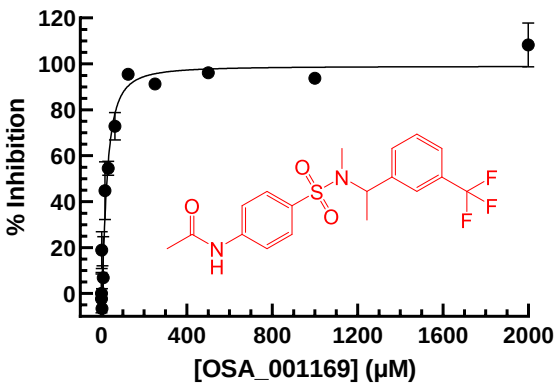
OSA_001153

IC50 = 299.1 ± 95.07 µM



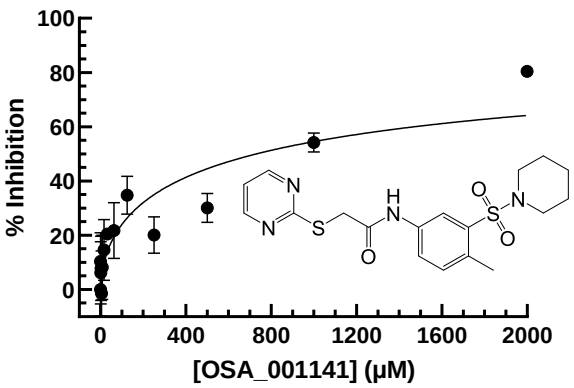
OSA_001169

IC50 = 25.37 ± 3.74 µM



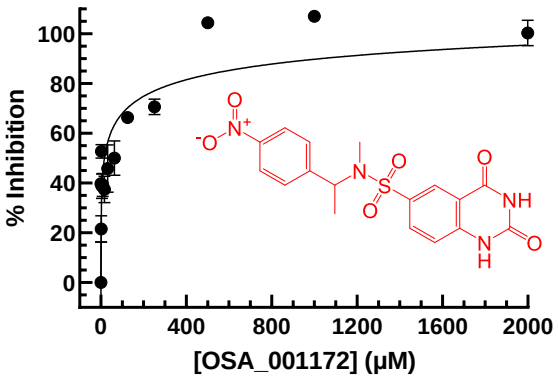
OSA_001141

IC50 = 729.9 ± 171.8 µM

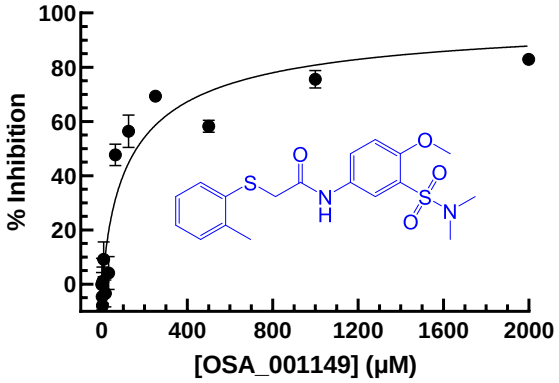


Characterization of inhibition of *P. aeruginosa* MurC by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Dose Response Curves (3)

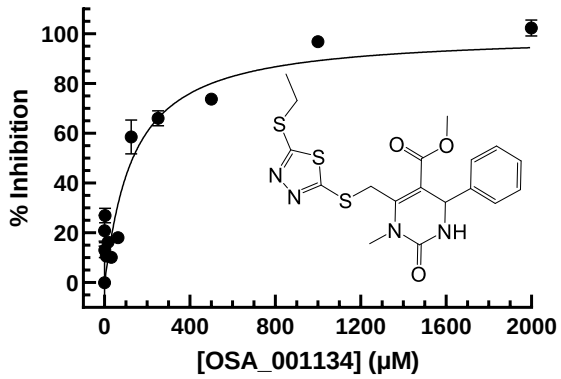
OSA_001172
IC50 = 73.82 ± 182.9 µM



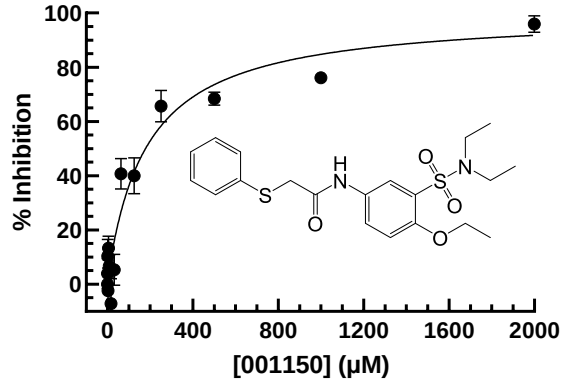
OSA_001149
IC50 = 140.1 ± 27.54 µM



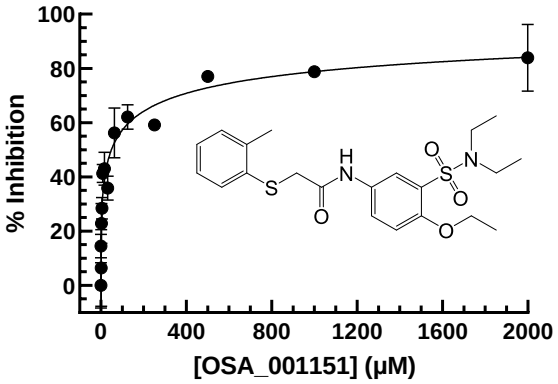
OSA_001134
IC50 = 132.0 ± 23.54 µM



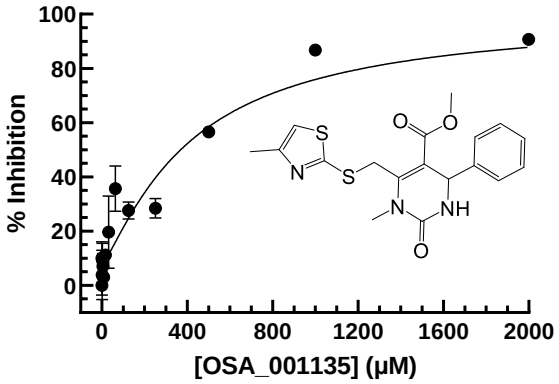
OSA_001150
IC50 = 184.0 ± 25.6 µM



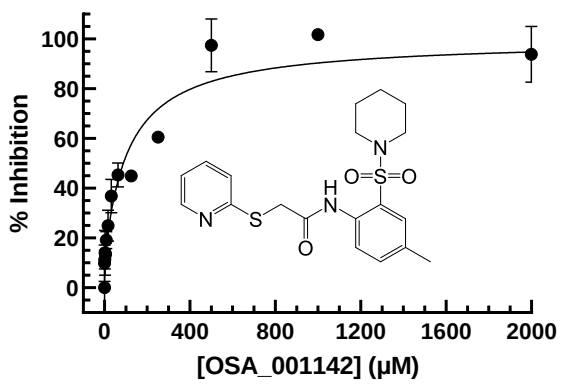
OSA_001151
IC50 = 80.00 ± 93.02 µM



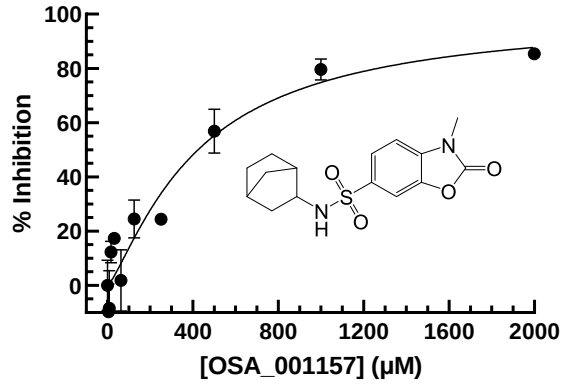
OSA_001135
IC50 = 422.3 ± 157.2 µM



OSA_001142
IC50 = 109.8 ± 17.5 µM



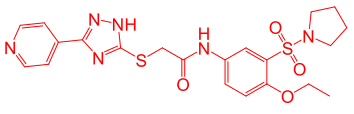
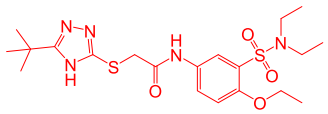
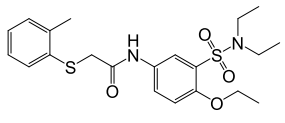
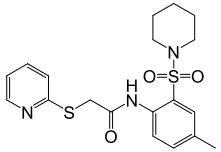
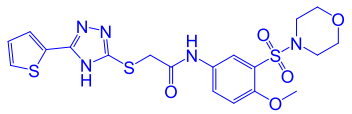
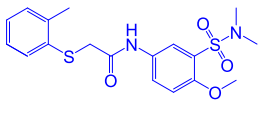
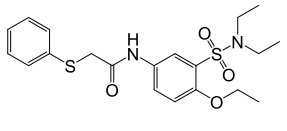
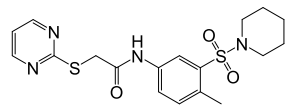
OSA_001157
IC50 = 423.9 ± 66.02 µM



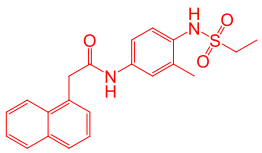
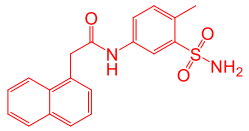
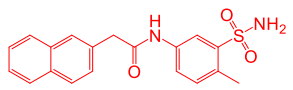
Inhibition of *P. aeruginosa* MurC by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: IC50 relationships within structurally similar inhibitors

IC50 (μM) ± SD (n = 3)

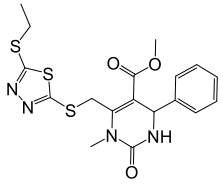
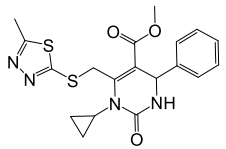
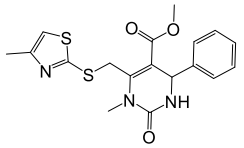
Acyl thio-N-Phenyl acetamides

22.1 ± 2.69	33.8 ± 4.38	80.00 ± 93.02	109.8 ± 17.5	130.9 ± 30.2	140.1 ± 27.54	184.0 ± 25.6	729.9 ± 171.8
							
OSA_001147	OSA_001145	OSA_001151	OSA_001142	OSA_001148	OSA_001149	OSA_001150	OSA_001141

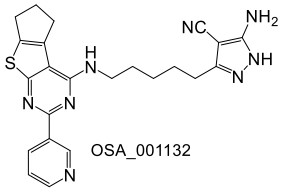
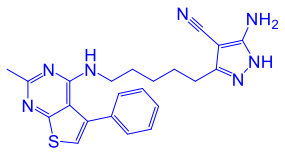
Napthyl phenyl sulfonamides

58.00 ± 6.20	244.5 ± 41.3	299.1 ± 95.07
		
OSA_001155	OSA_001156	OSA_001153

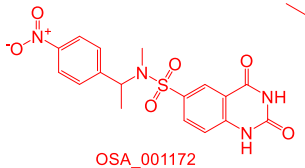
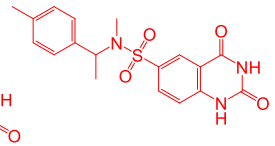
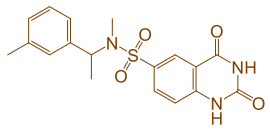
Thiadiazol phenyl pyrimidines

132.0 ± 23.54	238.6 ± 114.7	422.3 ± 157.2
		
OSA_001134	OSA_001136	OSA_001135

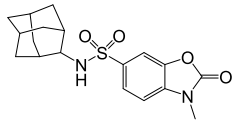
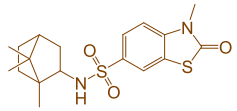
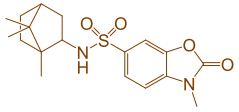
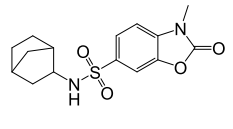
Amino pentyl pyrazoles

14.6 ± 1.33	25.5 ± 3.15
	
OSA_001132	OSA_001133

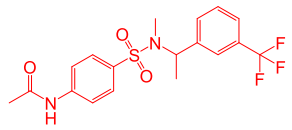
Tetrahydroquinazoline-6-sulfonamides

73.82 ± 182.9	124.3 ± 19.2	127.4 ± 17.2
		
OSA_001172	OSA_001164	OSA_001168

Acyl benzoxazole sulfonamides

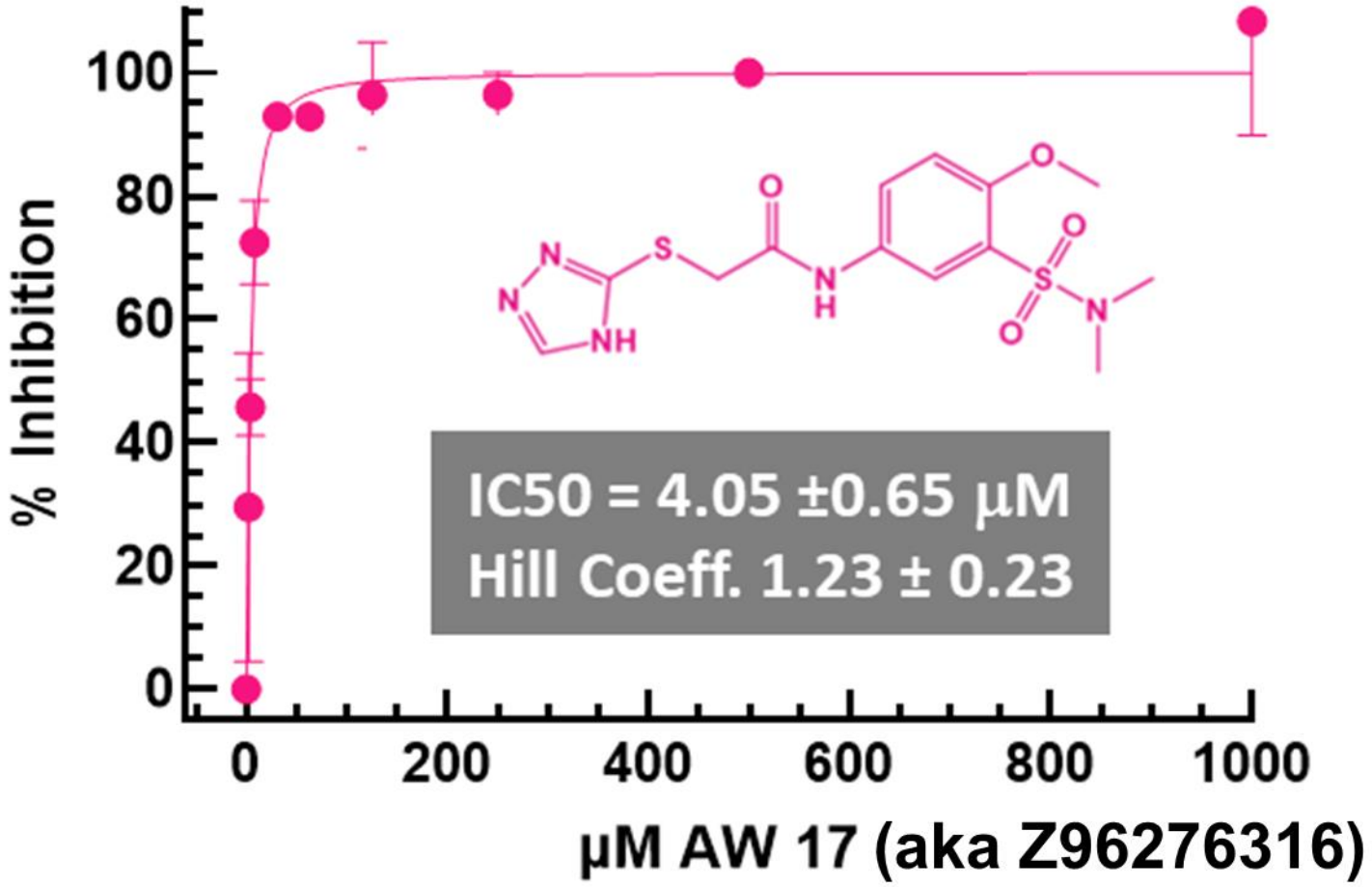
19.6 ± 18.6	38.0 ± 13.1	68.2 ± 15.2	423.9 ± 66.02
			
OSA_001159	OSA_001160	OSA_001167	OSA_001157

Sulfamoyl phenyl acetamides

25.3 0.237 ± 3.74

OSA_001169

Acyl thio-N-Phenyl acetamide previously identified in a previous screen of Atomwise compounds delivered in June 2021 and reported on 13-09-22

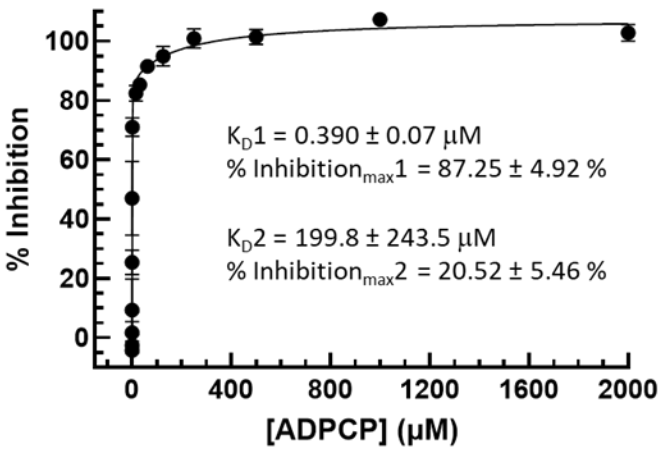
N-[3-(dimethylsulfamoyl)-4-methoxyphenyl]-2-(4H-1,2,4-triazol-3-ylsulfanyl)acetamide



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

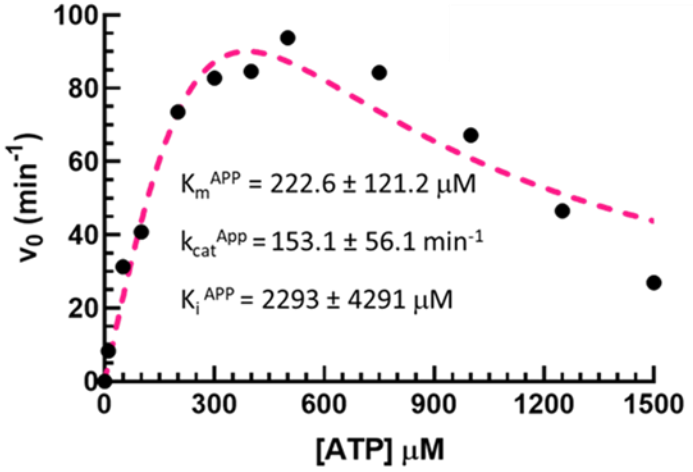
Inhibition of MurC by ADPCP

Biphasic two K_D values



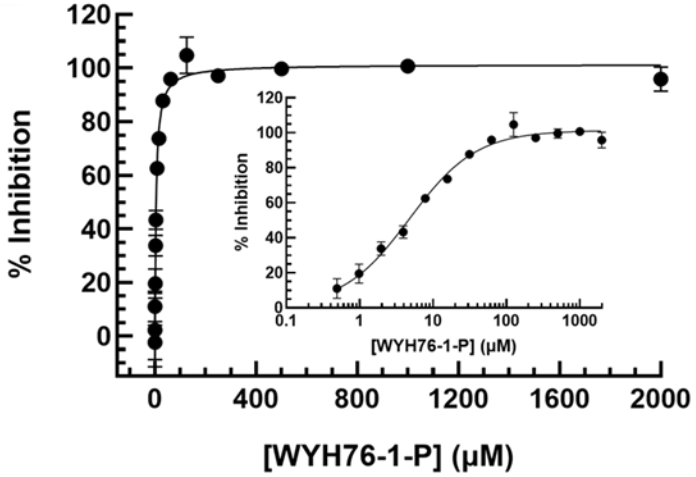
Dependence of MurC velocity on ATP concentration

Substrate Inhibition

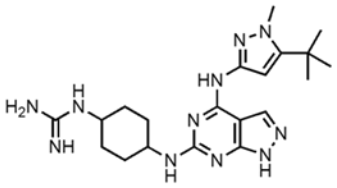


Inhibition of MurC by WYH76-1-P

Monophasic one KD value



WYH76-1-P



IC50 = 4.78 ± 0.46 μM
Hill Coefficient = 0.96 ± 0.08

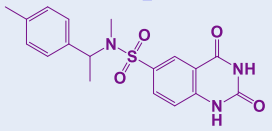
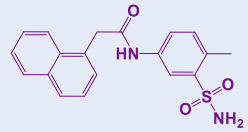
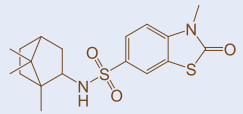
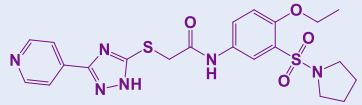
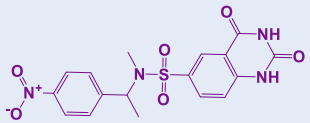
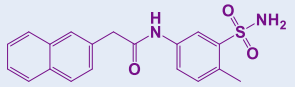
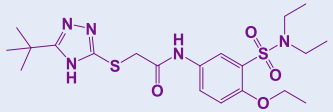
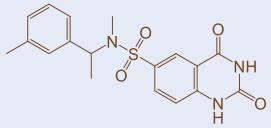
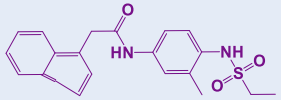
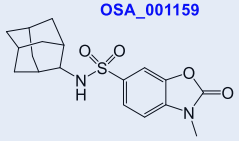
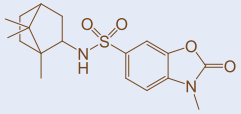
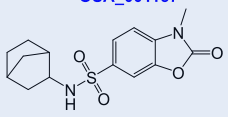
Data suggest that ADPCP mimics ATP in that it binds in two different ways to MurC, in contrast to the behaviour of the pyrazolopyrimidines

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 $MgCl_2$, 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)
OSA_001164 	95.8 ± 2.2	OSA_001156 	67.3 ± 8.6
OSA_001160 	77.4 ± 8.7	OSA_001147 	67.0 ± 4.0
OSA_001172 	72.1 ± 3.6	OSA_001153 	61.9 ± 2.1
OSA_001145 	71.7 ± 2.6	OSA_001168 	59.9 ± 7.3
OSA_001155 	69.4 ± 5.5	OSA_001159 	56.7 ± 1.7
OSA_001167 	69.1 ± 7.3	OSA_001157 	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.

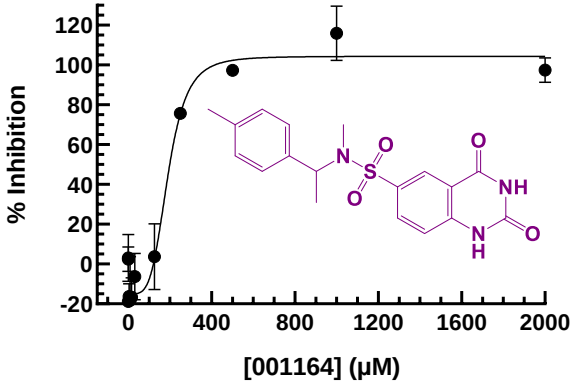
Characterization of inhibition of *P. aeruginosa* MurF by Atomwise hits from compound set delivered on the 23rd May and 25th July, 2023: Complete Data Set

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

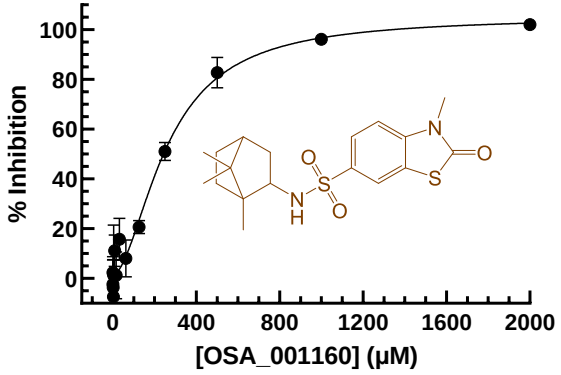
OSA_001164

IC50 = 190.4 ± 16.36 µM



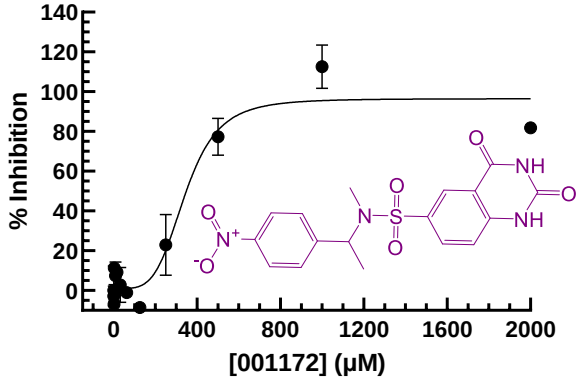
OSA_001160

IC50 = 261.0 ± 32.34 µM



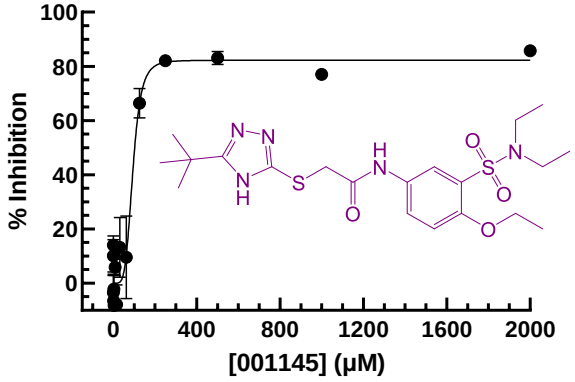
OSA_001172

IC50 = 343.9 ± 36.82 µM



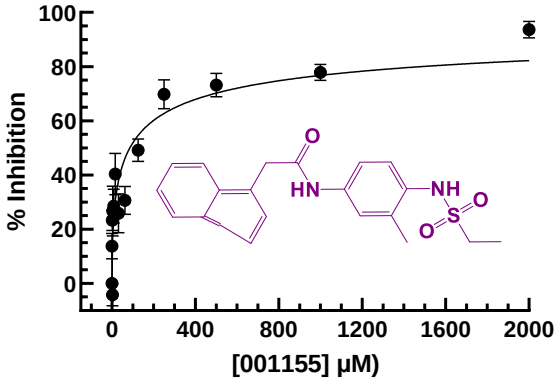
OSA_001145

IC50 = 93.05 ± 7.01 µM



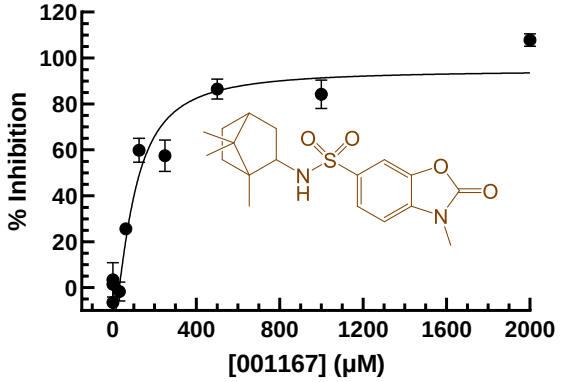
OSA_001155

IC50 = 85.54 ± 18.24 µM



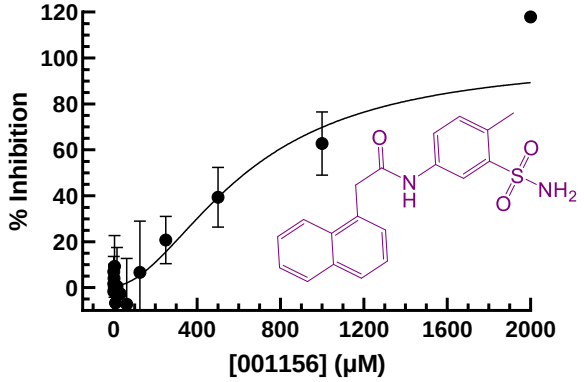
OSA_001167

IC50 = 99.41 ± 19.04 µM



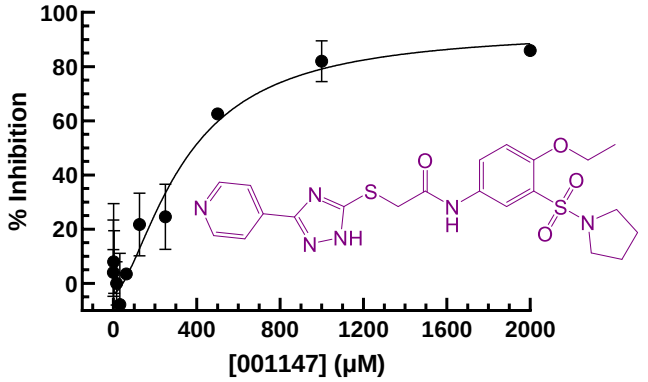
OSA_001156

IC50 = 639.2 ± 78.70 µM



OSA_001147

IC50 = 339.8 ± 87.50 µM

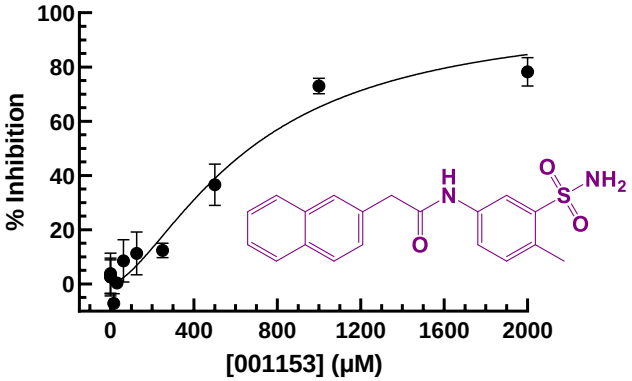


Maroon coloured compounds are >50% inhibitors of MurC, D, E and F, brown compounds are >50% inhibitors of MurC, D and F, black compounds are >50% inhibitors of MurC and MurF.

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)

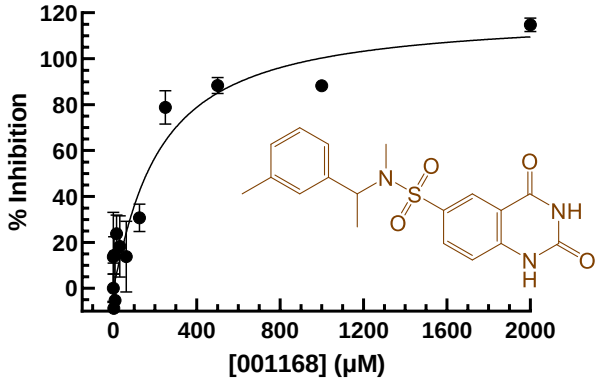
OSA_001153

IC50 = 668.3 ± 46.17 µM



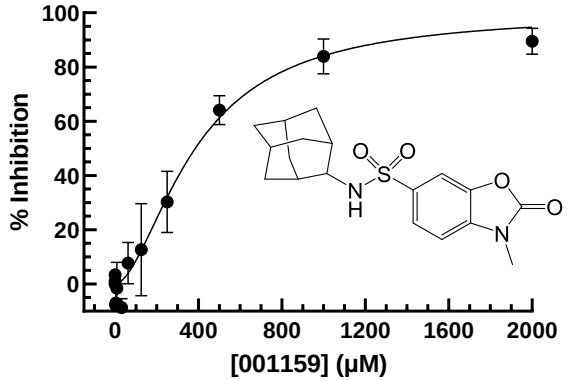
OSA_001168

IC50 = 216.8 ± 71.18 µM



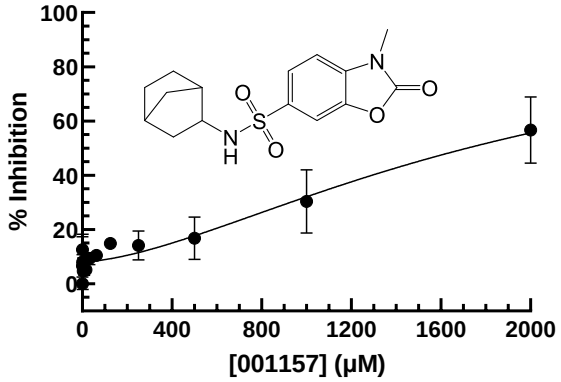
OSA_001159

IC50 = 384.3 ± 29.93 µM



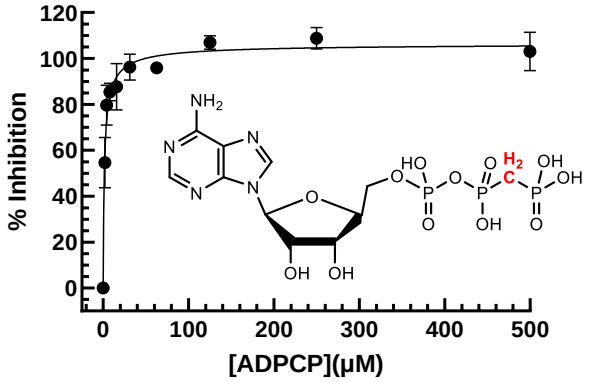
OSA_001157

IC50 = 1917 ± 176.9 µM



ADPCP

IC50 = 1.459 ± 0.319 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.

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 *Pseudomonas aeruginosa* 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

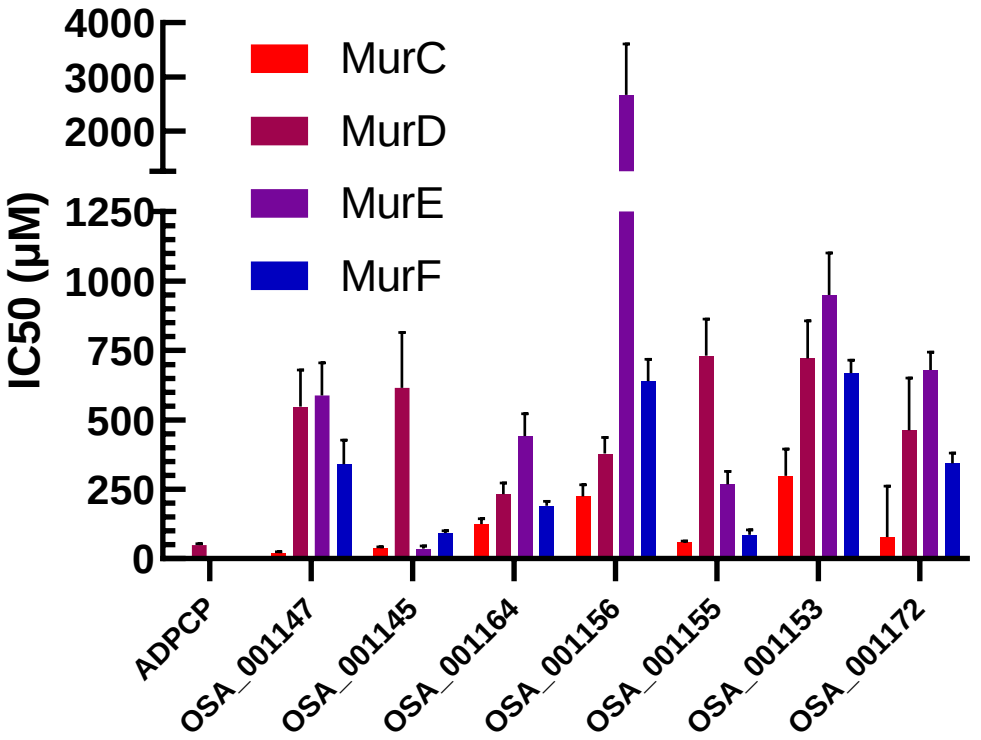
All data are mean ± SD for triplicate data. Gaps indicate that that particular inhibitor/ligase combination failed to register greater than 50 % inhibition at 0.5 mM OSA compound in the initial screen.

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

Corresponding IC50 determinations

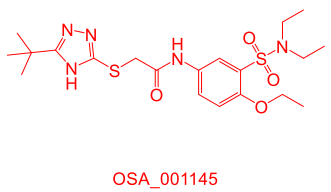


- Clearly, for all OSA compounds, exerting >50% inhibition in the initial screen at 0.5 mM, MurC is more susceptible to inhibition than MurD, E and F as determined by IC50.
- Of the seven OSA compounds that targeted all ligases, the IC50 values were clearly lowest for MurC (top right).
- Single, (MurC)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

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

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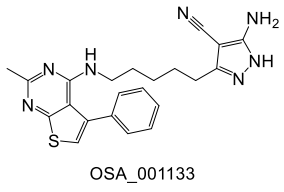
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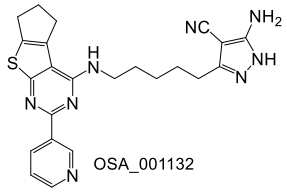
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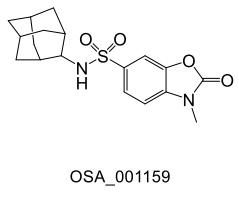
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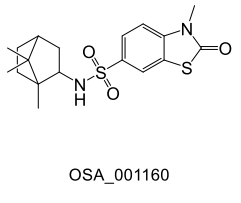
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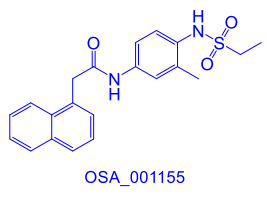
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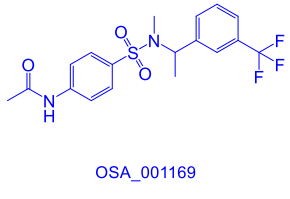
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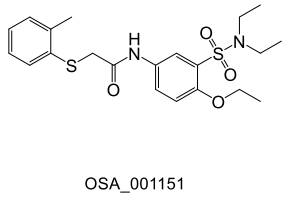
OSA_001151