Optimization of methionyl tRNA-synthetase inhibitors for treatment of *Cryptosporidium* infection

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Supplementary Data and Methods

Figure S1. Gel of purified CpMetRS showing the soluble (S), pure (P), and the size-exclusion chromatography fractions that were pooled (B11-B3). MW markers in kilodaltons.

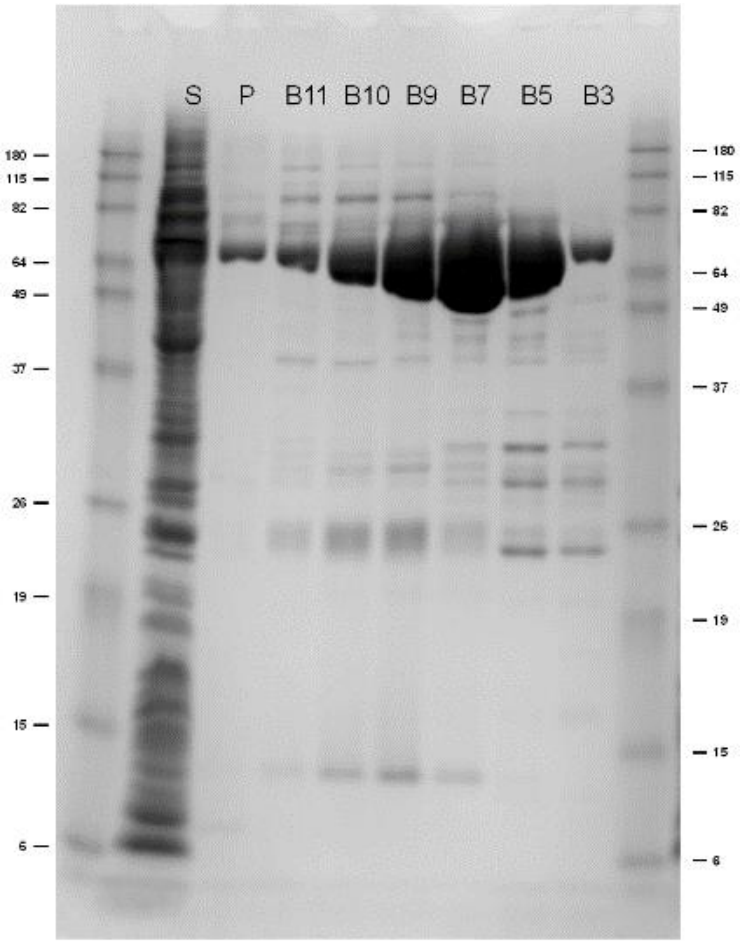


Figure S2. CpMetRS aminoacylation assay activity confirmation: bulk *E. coli* tRNA was titrated and disintegrations per minute (DPM) were measured.

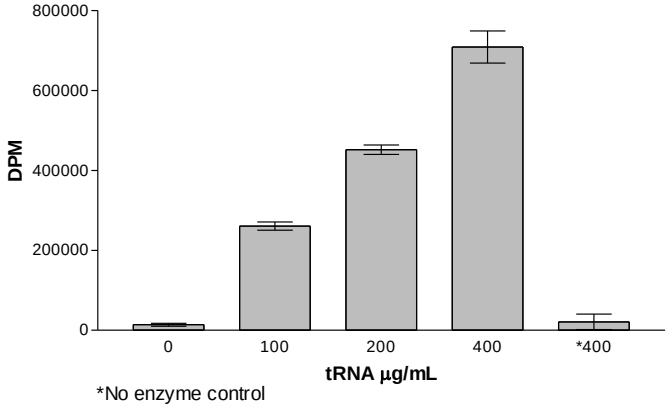
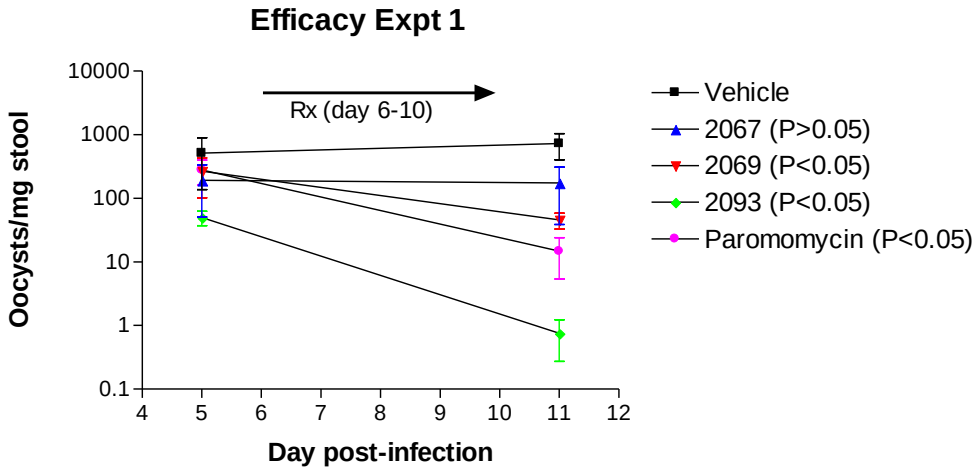


Figure S3. Efficacy of MetRS inhibitors in murine *Cryptosporidium parvum* (NSG) infection model. NOD SCID Gamma (NSG) mice, n=4 per group, were infected with oocysts on Day 0 and treated with test compounds (50 mg/kg PO BID), Paromomycin (2000 mg/kg PO QD), or vehicle (BID) on days 6-10 post-infection. Stool was collected on days 5 and 11 for quantitative PCR. P values relate to comparisons of parasite levels on day 11 between vehicle-treated and compound-treated groups.



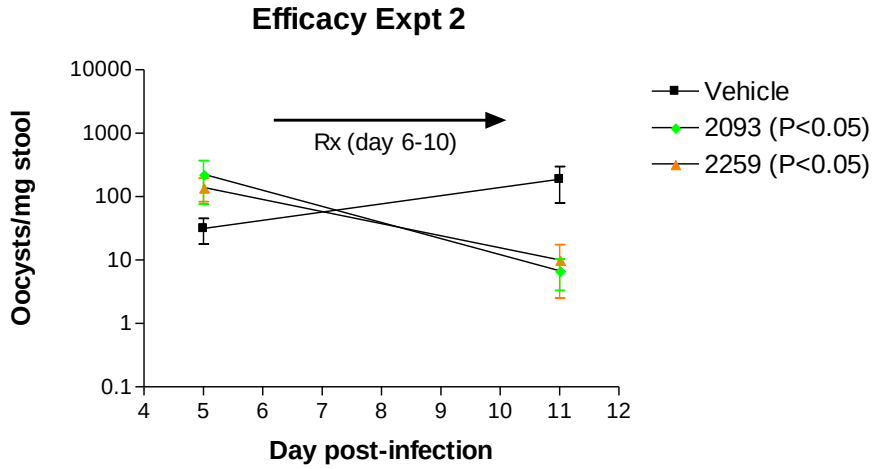


Table S1. Growth inhibitory activity of compound **2093** and control compounds against various *C. parvum* and *C. hominis* strains, and cytotoxicity against mammalian cell lines (HCT-8, HEK293, and HepG2).

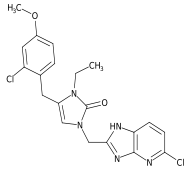
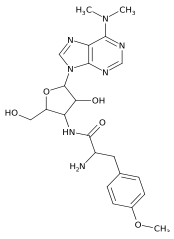
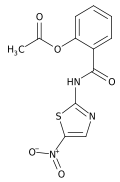
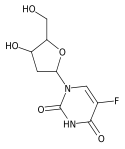
Molecule Name	Structure	Sterling Lab C. parvum Spot Count: EC50 (uM)	Sterling Lab C. parvum Spot Count: EC90 (uM)	Bunch Grass Farm C. parvum Spot Count: EC50 (uM)	Bunch Grass Farm C. parvum Spot Count: EC90 (uM)	C. hominis Spot Count: EC50 (uM)	C. hominis Spot Count: EC90 (uM)	Wild C. parvum Spot Count: EC50 (uM)	Wild C. parvum Spot Count: EC90 (uM)	HCT-8 Cell Count (Host cells infected with Sterling oocysts): CC50 (uM)	72H HEK293T Cytotoxicity: CC50 (uM)	72H HepG2 Cytotoxicity: CC50 (uM)
ChemID:2093		0.00645	0.0146	0.00919	0.022	0.0149	0.0616	0.0286	0.0495	> 25.0	37.5	26.2
Puromycin		0.133	0.585	0.15	0.363	0.291	0.626	0.23	1.98	0.674	0.392	0.421
Nitazoxanide		0.859	1.49	1.38	9.08	3.02	26.4	3.3	8.92	> 6.67	72.8	38.4
Floxuridine		0.00511	0.0268	0.00952	0.0284	0.0242	0.119	0.0272	0.0471	0.00884	> 1.60	0.00384

Table S2. Ames test for **2093.**

Table 1 Reversion test in *S. typhimurium* without S9 mix

Test article	Dose (µg/plate)	Number of revertant colonies / plate (mean) TA100	TA98
DMSO	0	94	33
		116	15
		103	22
		90 (101)	31 (25)
2093	78.1	101	21
		113 (107)	19 (20)
	156.3	79	29
		103 (91)	24 (27)
	312.5	73	26
		80 (77)	26 (26)
	625	81	24
		86 (84),P	29 (27),P
	1250	114	20
		85 (100),P,K	21 (21),P,K
	2500	103	29
		90 (97),P,K	12 (21),P,K
	5000	106	26
		96 (101),P,K	16 (21),P,K
AF-2	0.01	411	NT
		425 (418)	
	0.1	NT	401
			360 (381)

P: precipitation; K: growth inhibition; NT: not tested
DMSO: dimethyl sulfoxide; AF-2: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide

Table 2 Reversion test in *S. typhimurium* with S9 mix

Test article	Dose (µg/plate)	Number of revertant colonies / plate (mean) TA100	TA98
DMSO	0	106	22
		107	34
		109	31
		114 (109)	29 (29)
2093	78.1	96	36
		106 (101)	22 (29)
	156.3	143	41
		113 (128)	20 (31)
	312.5	137	52
		134 (136)	34 (43)
	625	113	33
		113 (113)	45 (39)
	1250	127	33
		96 (112),P,K	22 (28),P
	2500	111	24
		96 (104),P,K	14 (19),P
	5000	95	24
		107 (101),P,K	21 (23),P
2AA	1	589	749
		620 (605)	840 (795)

P: precipitation; K: growth inhibition
DMSO: dimethyl sulfoxide; 2AA: 2-aminoanthracene

Table S3. Micronucleus test for **2093**.

Group.	Test article	Concen-tration (mg/mL)	Treatment conditions	Cell number[a] well 1 (cells/mL)	Cell number[a] well 2 (cells/mL)	Cell number[a] mean (cells/mL)	Population doubling	Relative population doubling (%)	Precipitation at the start of treatment	Precipitation at the end of treatment
1	DMSO	10 µL/mL	3 h	258,800	252,520	255,660	1.35	100.0	S	S
2	2093	22.8	+S9 mix	243,560	262,280	252,920	1.34	98.9	S	S
3		29.6		236,920	245,680	241,300	1.27	93.8	S	S
4		38.5		202,440	209,520	205,980	1.04	77.0	S	S
5		50.1		181,160	183,680	182,420	0.87	64.0	S	S
6		65.1		176,680	175,960	176,320	0.82	60.4	S	S
7		84.6		144,480	136,720	140,600	0.49	36.3	S	S
8		110		80,000	81,800	80,900	-0.31	-22.6	P	S
9	CP	6					ND			
10	DMSO	10 µL/mL	24 h	312,240	312,440	312,340	1.64	100.0	S	S
11	2093	10.4	-S9 mix	239,720	253,240	246,480	1.30	79.2	S	S
12		13.5		255,400	251,000	253,200	1.34	81.6	S	S
13		17.5		216,800	208,480	212,640	1.09	66.2	S	S
14		22.8		208,160	193,120	200,640	1.00	61.1	S	S
15		29.6		176,640	165,320	170,980	0.77	47.1	S	S
16		38.5		133,120	125,520	129,320	0.37	22.6	S	S
17		50.1		105,280	99,600	102,440	0.03	2.1	S	S
18	COL	0.01					ND			

(a) Initial cell number = 100,000 cells/mL

COL=colcemid, CP=cyclophosphamide, DMSO=dimethyl sulfoxide

ND=not determined, P=precipitation, S=soluble

Group.	Test article	Concen-tration (µg/mL)	Treatment conditions	Relative population doubling (%)	Micronucleated cells well 1	well 2	total[1)]	(%)	
1	DMSO	10 µL/mL	3 h	100.0	15	13	28	(0.70)	
2	2093	22.8	+S9 mix	98.9					
3		29.6		93.8			ND		
4		38.5		77.0					
5		50.1		64.0	11	11	22	(0.55)	
6		65.1		60.4	10	8	18	(0.45)	
7		84.6		36.3	16	8	24	(0.60)	
8		110		-22.6			ND		
9	CP	6		ND	29	44	73	(1.83)	**
10	DMSO	10 µL/mL	24 h	100.0	15	15	30	(0.75)	
11	2093	22.8	-S9 mix	79.2			ND		
12		29.6		81.6					
13		38.5		66.2	14	20	34	(0.85)	
14		50.1		61.1	12	12	24	(0.60)	
15		65.1		47.1	13	13	26	(0.65)	
16		84.6		22.6			ND		
17		110		2.1					
18	COL	0.01		ND	37	37	74	(1.85)	**

1) A total of 4,000 cells were counted

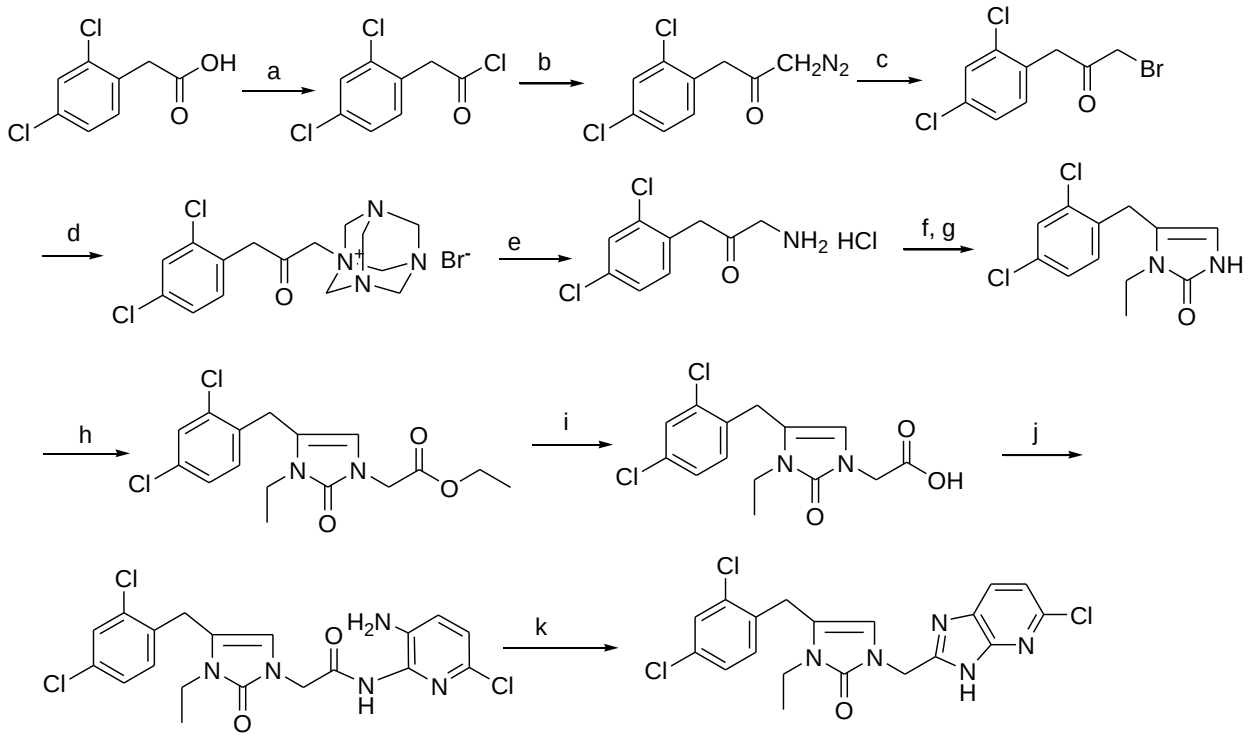
COL=colcemid, CP=cyclophosphamide, DMSO=dimethyl sulfoxide, ND=not determined

**: $p<0.01$, Fisher's exact test

Chemistry Methods:

Synthesis of **2067**, **2069**, and **2091** followed the general procedures **1** as described for compound **2062** reported earlier (1) and is presented for **2067** below:

Scheme S1



2067

-Reagents and conditions (a) oxalyl chloride, DMF, DCM; (b) trimethylsilyldiazomethane, THF/MeCN; (c) HBr; (d) hexamethylenetetramine, DCM; (e) ethanol/HCl; (f) ethyl isocyanate, DCM; (g) TFA; (h) ethyl bromoacetate, K_2CO_3, ; (i) LiOH, ethanol/water; (j) EDC, 6-floropyridine-2,3-diamine, pyridine; (k) HOAc, microwave irradiation.

General Procedure 1:

To a solution of 2,4-dichlorophenylacetic acid (308 mg, 1.5 mmol) in dry THF (6 ml) was added dropwise oxalyl chloride (0.21 ml, 2.3 mmol) and one drop of dry DMF at room temperature. The reaction mixture was stirred at room temperature overnight and the solvent was completely removed in vacuo to obtain 2,4-dichlorophenylacetyl chloride. 2, 4-dichlorophenylacetyl chloride also can be made by microwave irradiation of dichlorophenylacetic acid (308 mg, 1.5 mmol) in 10 ml of thionyl chloride at 80 °C for 1 hour and the solvent was completely removed in vacuo.

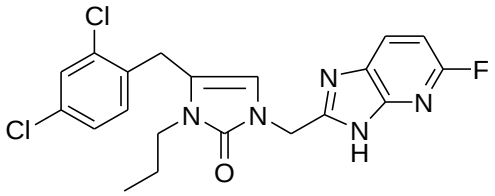
The residue was dissolved in anhydrous MeCN /THF (8 ml/8ml) and dropwise added 5.25 ml (3.15 mmol) of a 0.6 M solution or 1.58 ml of 2.0 M of trimethylsilyldiazomethane in hexane at 0°C. The mixture was stirred at 0°C for 1h and overnight at room temperature. The mixture was cooled to 0°C and 0.5ml of 48% HBr was added dropwise (gas evolution). The solution was stirred at 0°C for 15 min and room temperature for 2h. After concentrated in vacuo, the residues was dissolved with EtOAc and washed successively with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc), giving bromo-3-(2,4-dichlorophenyl)acetone in 71% yield (299 mg).

To the solution of bromo-3-(2,4-dichlorophenyl)acetone (241 mg, 0.859 mmol) in DCM was added 140 mg of hexamethylenetetramine (0.945 mmol). The mixture was vigorously stirred at room temperature overnight. After cooled down, the white solid is filtered and washed with cold DCM to obtain 307 mg quaternary ammonium HBr salt. The white solid was suspended in 10 ml of EtOH and 2 ml of concentrated HCl was added to dissolve. The mixture was refluxed for 10 h and concentrated in vacuo to give 1-amino-3-(2,4-dichlorophenyl)propan-2-one as a white salt, which was used in the next reaction without purification.

The half of above salt in 20 ml of anhydrous methylene chloride at 0 °C was neutralized by adding DIPEA. The solution was added 25 µl of ethyl isocyanate (0.51 mmol). The mixture was stirred at 0 °C for 30min and room temperature for 3 h. Then 1 ml of TFA was added and the mixture was stirred at room temperature for 50 min. The mixture was concentrated and diluted with EtOAc and washed successively with saturated $NaHCO_3$, and then brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (DCM/MeOH), yielding 5-(2,4-dichlorobenzyl)-1-ethyl-1H-imidazol-2(3H)-one (67 mg). To a solution of 5-(2,4-dichlorobenzyl)-1-ethyl-1H-imidazol-2(3H)-one (67 mg, 0.246 mmol) in 8 ml anhydrous DMF was added 82 µl (0.738 mmol) of ethyl bromoacetate and 102 mg of K_2CO_3 (0.738 mmol). The mixture was stirred at room temperature overnight. After the reaction solvent was evaporated, the residues was dissolved with EtOAc and washed successively with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (dichloromethane/methanol) to obtain ethyl 2-(5-(2,4-dichlorobenzyl)-1-ethyl-1,2-dihydro-2-oxoimidazol-3-yl)acetate in 90% yield (79 mg, 0.22 mmol).

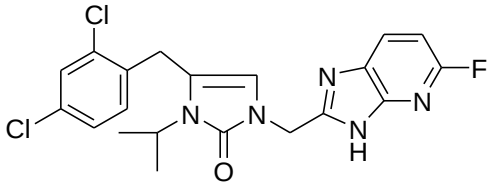
Ethyl 2-(5-(2,4-dichlorobenzyl)-1-ethyl-1,2-dihydro-2-oxoimidazol-3-yl)acetate (40 mg, 0.11 mmol) was added in 2 ml of ethanol and 4 ml of water and LiOH (11 mg, 0.9 mml). The solution was stirred for 50 min at room temperature and acidified with 0.2 N hydrochloric acid. After, the mixture was dried completely in vacuo. The residue

dissolved in 3 ml of pyridine was added 6-chloropyridine-2,3-diamine (0.13 mmol) and EDC hydrochloride (33 mg, 0.17mmol). The mixture was stirred at room temperature overnight. After the solvent was removed on the rotary evaporator, the residue was dissolved in EtOAc (30ml) and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel to give 2-(5-(2,4-dichlorobenzyl)-1-ethyl-1,2-dihydro-2-oxoimidazol-3-yl)-N-(3-amino-6-chloropyridin-2-yl)acetamide. 2-(5-(2,4-dichlorobenzyl)-1-ethyl-1,2-dihydro-2-oxoimidazol-3-yl)-N-(3-amino-6-chloropyridin-2-yl)acetamide was dissolved in 2 ml of acetic acid and microwave irradiated at 125 °C for 1h. After the solvent was removed on the rotary evaporator, the residue was purified by flash column chromatography (DCM/MeOH), giving 27 mg of **2067** in 58% yield. ^{1}H NMR (500 MHz, MeOD) δ 7.86 (d, *J* = 12.2 Hz, 1H), 7.44 (s, 1H), 7.32-7.16 (m, 3H), 6.11 (s, 1H), 5.00 (s, 2H), 3.87 (s, 2H), 3.59 (q, *J* = 7.2 Hz, 2H), 1.08 (t, *J* = 8.6 Hz, 3H). LC/MS: (ESI) (M +H)$^+$= 437.6.



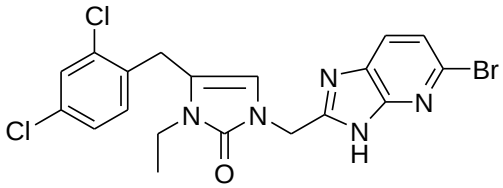
2069

2069 was synthesized using propyl isocyanate following General Procedure 1. ^{1}H NMR (500 MHz, MeOD) δ 8.12 – 7.77 (m, 1H), 7.43 (s, 1H), 7.24 (s, 2H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.10 (s, 1H), 6.05 (s, 1H), 4.98(s, 2H), 3.77 (s, 2H), 3.85 (s, 3H) ,3.61 – 3.39 (m, 2H), 1.53 – 1.48 (m, 2H), 0.83 (t, *J* = 7.4 Hz, 3H). LC/MS: (ESI) (M +H)$^+$= 435.3.



2080

2080 was synthesized using isopropyl isocyanate following General Procedure 1. ^{1}H NMR (500 MHz, MeOD) δ 8.03 (m, 1H), 7.48 (s, 1H), 7.30 (m, 2H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.16 (s, 1H), 5.03 (s, 2H), 4.09 (m, 1H), 3.92 (s, 2H), 1.41 (d, *J* = 6.8 Hz, 6H) LC/MS: (ESI) (M +H)$^+$= 435.4.

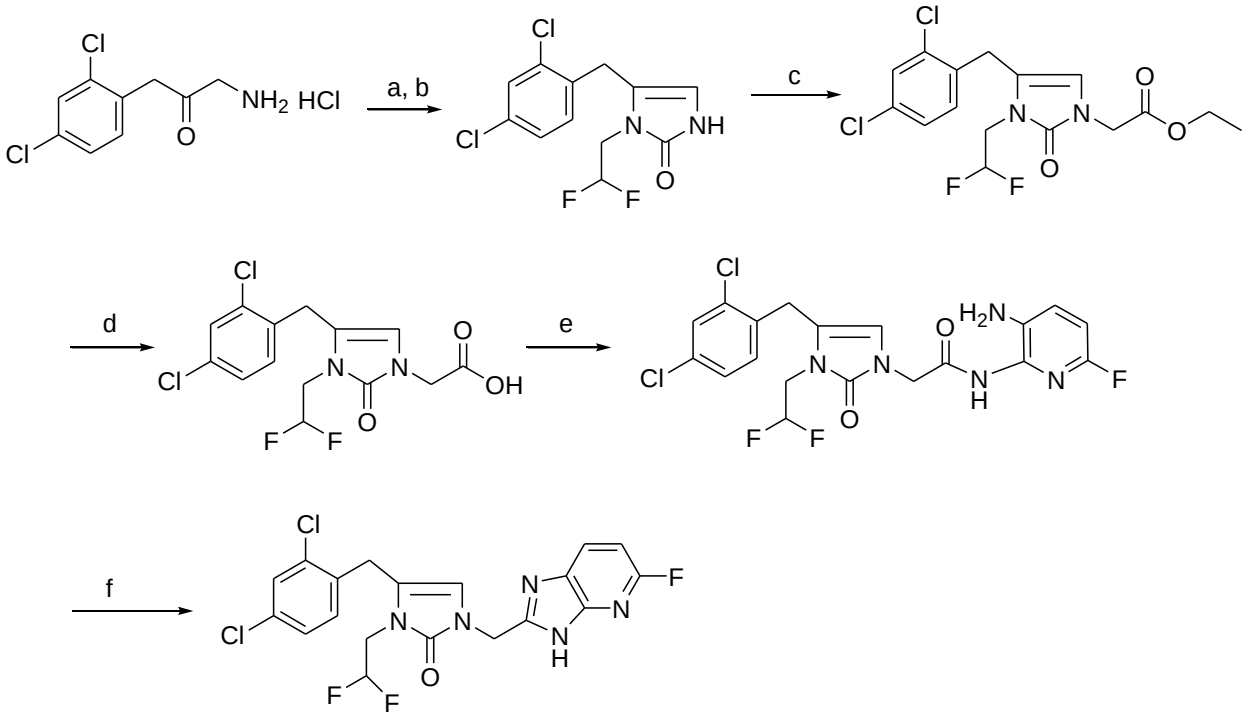


2091

2091 was synthesized using 6-bromopyridine-2,3-diamine following General Procedure 1. ^{1}H NMR (500 MHz, MeOD) δ 7.86 (d, *J* = 8.3 Hz, 1H), 7.50 (s, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.31 (s, 1H), 6.17 (s, 1H), 5.06 (s, 2H), 3.93 (s, 2H), 3.67-3.63 (m, 2H), 1.15 (t, *J* = 7.2 Hz, 3H). LC/MS: (ESI) $(M+H)^{+}$= 482.4.

Synthesis of compounds **2138**, **2139**, **2207**, **2240**, **2258**, and **2259** followed the general procedures 1 and 2 as described for **2139** below.

Scheme S2



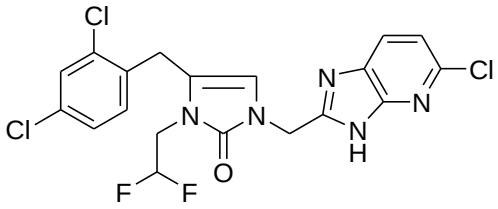
2139

Reagents and conditions (a) triphosgen, DIPEA, DCM, 2,2-difluoroethanamine; (b)TFA; (c) ethyl bromoacetate, K_2CO_3 ; (d) LiOH, ethanol/water; (e) EDC, 6-fluoropyridine-2,3-diamine, pyridine; (f) HOAc, microwave irradiation.

General Procedure 2:

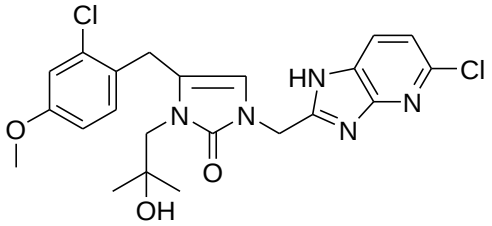
To an ice-cooled suspension of 1-amino-3-(2,4-dichlorophenyl)propan-2-one HCl salt (25.4 mg 0.1 mmol, from the general procedure 1 above) in anhydrous methylene chloride (10 mL), DIPEA (50 μL) and triphosgene (5.9 μl, 0.035 mmol) were added. After the mixture was stirred at 0 °C for 1 h, 2,2-difluoroethanamine (0.11 mmol) was added. The mixture was stirred at 0°C for 30 min and room temperature for 1 h. Then 1 ml of TFA was added and the mixture was stirred at room temperature for 50 min. After most solvent was removed, the mixture was diluted with EtOAc and washed successively with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash column chromatography (DCM/MeOH), yielding 5-(2,4-dichlorobenzyl)-1-(2,2-difluoroethyl)-1H-imidazol-2(3H)-one (15 mg). 5-(2,4-dichlorobenzyl)-1-ethyl-1H-imidazol-2(3H)-one (15 mg, 0.049 mmol) was dissolved in 10 ml anhydrous acetonitrile and treated with 16.3 μl (0.147 mmol) of ethyl bromoacetate and potassium carbonate (20.3 mg, 0.147 mmol). The mixture was refluxed overnight. After the reaction solvent was evaporated, the residues was dissolved with EtOAc and washed successively with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (dichloromethane/methanol) to obtain ethyl 2-(5-(2,4-dichlorobenzyl)-1-(2,2-difluoroethyl)-1,2-dihydro-2-oxoimidazol-3-yl)acetate in 90% yield (17.3 mg, 0.044 mmol).

Ethyl 2-(5-(2,4-dichlorobenzyl)-1-(2,2-difluoroethyl)-1,2-dihydro-2-oxoimidazol-3-yl)acetate (17.3 mg, 0.044 mmol) was added in 1 ml of ethanol and 3 ml of water, mixed with LiOH (1.1 mg, 0.176 mml) and stirred for 50 min at room temperature. The solution was acidified with 0.2 N hydrochloric acid and the solvent completely removed in vacuo. The residue was dissolved in 3 ml of pyridine, then 6-fluoropyridine-2,3-diamine (0.25 mmol) and EDC hydrochloride (52 mg, 0.27 mmol) were added. The mixture was stirred at room temperature overnight. The solvent was removed on a rotary evaporator. The residue was dissolved in EtOAc (30 ml) and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel to give 2-(5-(2,4-dichlorobenzyl)-1-(2,2-difluoroethyl)-1,2-dihydro-2-oxoimidazol-3-yl)-N-(3-amino-6-fluoropyridin-2-yl)acetamide. 2-(5-(2,4-dichlorobenzyl)-1-(2,2-difluoroethyl)-1,2-dihydro-2-oxoimidazol-3-yl)-N-(3-amino-6-fluoropyridin-2-yl)acetamide was dissolved in 2ml of acetic acid and the solution was microwave irradiated at 125 ºC for 1 h. After the solvent was removed on a rotary evaporator, the residue was purified by flash column chromatography (DCM/MeOH), producing **2139** in 41% yield (8.2 mg). ^{1}H NMR (500 MHz, MeOD) δ 7.86 (d, *J* = 8.3 Hz, 1H), 7.50 (s, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.31 (s, 1H), 6.17 (s, 1H), 5.06 (s, 2H), 3.93 (s, 2H), 3.67-3.63 (m, 2H), 1.15 (t, *J* = 7.2 Hz, 3H). LC/MS: (ESI) $(M+H)^+$= 457.3.



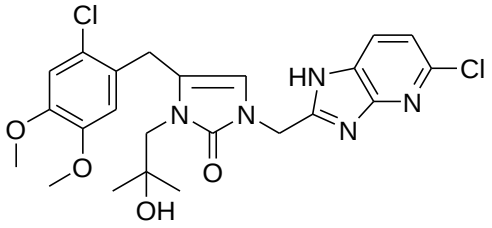
2138

2138 was synthesized using 6-chloropyridine-2,3-diamine following General Procedure 2. ^{1}H NMR (500 MHz, MeOD) δ 7.92 (d, *J* = 8.5 Hz, 1H), 7.50 (s, 1H), 7.30 (m, 3H), 6.18-6.07 (m, 2H), 5.06 (s, 2H), 4.03 (m, 2H), 3.94 (s, 2H). LC/MS: (ESI) $(M+H)^{+}$= 473.6.



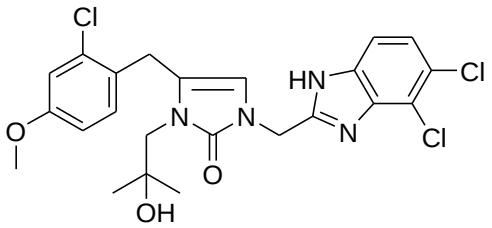
2207

2207 was synthesized using 2-(2-chloro-4-methoxyphenyl)acetic acid and 1-amino-2-methylpropan-2-ol following General Procedure 1 and 2. ^{1}H NMR (300 MHz, MeOD) δ 7.93 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 6.99 (s, 1H), 6.86 (d, *J* = 6.2 Hz, 1H), 6.02 (s, 1H), 5.08 (s, 2H), 3.98 (s, 2H), 3.80 (s, 3H), 3.66 (s, 2H), 1.26(s, 6H). LC/MS: (ESI) $(M+H)^{+}$= 477.5.



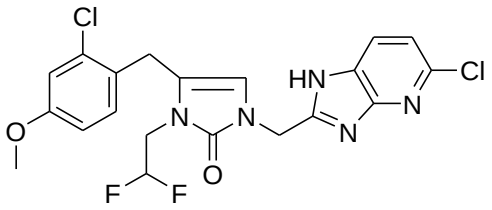
2240

2240 was synthesized using 2-(2-chloro-4,5-dimethoxyphenyl)acetic acid,1-amino-2-methylpropan-2-ol and 6-chloropyridine-2,3-diamine following General Procedure 1 and 2. ^{1}H NMR (500 MHz, MeOD) δ 7.92 (d, *J* = 8.0 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 6.97 (s, 1H), 6.85 (s, 1H), 6.03 (s, 1H), 5.07 (s, 2H), 3.96 (s, 2H), 3.81 (s, 3H), 3.74 (s, 3H), 3.66 (s, 2H), 1.24(s, 6H). LC/MS: (ESI) $(M+H)^{+}$= 507.3.



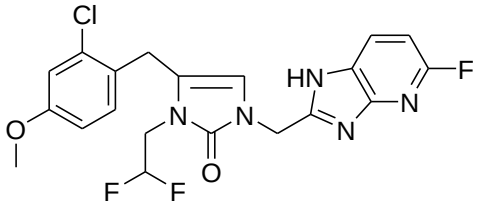
2242

2242 was synthesized using 2-(2-chloro-4-methoxyphenyl)acetic acid, 1-amino-2-methylpropan-2-ol and 3,4-dichlorobenzene-1,2-diamine following General Procedure 1 and 2. ^{1}H NMR (500 MHz, MeOD) δ 7.44 (m, *J* = 8.4 Hz, 1H), 7.38 (d, *J* = 8.5 Hz, 1H), 7.19 (d, *J* = 8.5 Hz, 1H), 6.99 (s, 1H), 6.85 (d, *J* = 9.3 Hz, 1H), 6.00 (s, 1H), 5.04 (s, 2H), 3.96 (s, 2H), 3.79 (s, 3H), 3.65 (s, 2H), 1.23(s, 6H); LC/MS: (ESI) $(M+H)^{+}$= 510.7.



2258

2258 was synthesized using 2-(2-chloro-4-methoxyphenyl)acetic acid and 6-chloropyridine-2,3-diamine following General Procedure 1 and 2. ^{1}H NMR (500 MHz, MeOD) δ 7.93 (d, *J* = 8.3 Hz, 1H), 7.30 (d, *J* = 8.4 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 7.00 (s, 1H), 6.86 (d, *J* = 8.1 Hz, 1H), 6.15-5.93 (m, 2H), 5.06 (s, 2H), 4.03 (m, 2H), 3.88 (s, 2H), 3.79 (s, 3H). LC/MS: (ESI) $(M+H)^{+}$= 469.4.



2259

2259 was synthesized using 2-(2-chloro-4-methoxyphenyl)acetic acid following General Procedure 1 and 2. ^{1}H NMR (500 MHz, MeOD) δ 8.03 (t, *J* = 7.2 Hz, 1H), 7.21 (d, *J* = 8.5 Hz, 1H), 6.99 (s, 1H), 6.94 (d, *J* = 8.6 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.22-5.69 (m, 2H), 5.05 (s, 2H), 4.03 (m, 2H), 3.87 (s, 2H), 3.78 (s, 3H). LC/MS: (ESI) $(M+H)^{+}$= 452.7.

References for Supplementary Materials.

1. Faghih O, Zhang Z, Ranade RM, Gillespie JR, Creason SA, Huang W, Shibata S, Barros-Alvarez X, Verlinde C, Hol WGJ, Fan E, Buckner FS. 2017. Development of Methionyl-tRNA Synthetase Inhibitors as Antibiotics for Gram-Positive Bacterial Infections. Antimicrob Agents Chemother 61.