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Parallel Hit Progression Strategy Identifies Improved Small Molecule Inhibitors of the Malaria Purine Uptake Transporter that Inhibit *Plasmodium falciparum* Parasite Proliferation

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Supplementary Table S1. Small molecule screening data

Category	Parameter	Description
Assay	Type of assay	Cell-based – *Saccharomyces cerevisiae*
	Target	PfENT1 – *Plasmodium falciparum* Equilibrative Nucleoside Transporter Type 1
	Primary measurement	Yeast cell growth (OD_{620})
	Key reagents	test compounds, 5-fluorouridine (5-FUrd), yeast cells, SDM growth media
	Assay protocol	1) Add 0.08 µl of test compound (10 mM stock in 100% DMSO) to test wells 2) Yeast grown overnight in SDM media diluted to OD_{620} = 0.05 with SDM media. 3) Add 5-FUrd to yeast cell suspension to a final concentration of 60 µM 5-FUrd 4) Add 8 µl cells (~8,000 cells) to each well; final concentrations: 10 µM test compound, 60 µM 5-FUrd, 1% DMSO v/v, 0.5 mM CHAPS in SDM media 5) Plates spun at 500 rpm for 1-2 min and equilibrated at room temperature (RT) for 30 min 6) Incubate for 16 h, 30 ^{o}C in humidified incubator 7) Plates were removed from the incubator and allowed to equilibrate at RT for at least 30 min 8) Read OD_{620}
Library	Library size	1,792,272
	Library composition	GSK Small Molecule Chemical Library
	Source	GlaxoSmithKline
Screen	Format	1536-well, round-well, flat-bottom Corning #3893
	Concentration(s) tested	10 µM

	Plate controls	Minimum: no test compound + 60 μM 5-FUrd +1% DMSO (v/v) Maximum: no 5-FUrd +1% DMSO (v/v)
	Reagent/ compound dispensing system	ThermoFisher Multidrop Combi cat# 5840399
	Detection instrument and software	Perkin Elmer Envision Model# 2102
	Assay validation/QC	Every test plate contained positive and negative controls in the outer two columns; for 1350 plates, average Z'-score = 0.70 $\pm$ 0.05, average S/B = 5.3 $\pm$ 1
	Correction factors	None
	Normalization	Well OD_{620} were normalized as a percentage of the maximum values in the control wells
Post-HTS analysis	Initial Hit criteria	Compounds with activity > 9.1% of maximal growth, >3 SD above the mean of the sample population
	Hit rate	3.9%
	Hit criteria for extensive analysis	Compounds with activity > 28% of maximal growth, $\geq$10 SD above the mean of the sample population (N=4104), plus 190 compounds with activity between 3 and 10 SD above the mean of the sample population were selected based on chemical scaffold diversity
	Hit rate > 10 SD	0.2%
	Additional assay(s)	Concentration-responses in 5-FUrd primary growth-rescue assay, orthogonal adenosine-dependent growth inhibition assay, [^{3}H]adenosine uptake inhibition assay, HepG2 cell cytotoxicity assay, *P. falciparum* cytotoxicity assay

Confirmation of hit purity and structure	Fresh compound was purchased directly from Enamine or ChemDiv. Purity and structure were confirmed by NMR

Supplementary Table S2. XC_{50} values for various assays performed on 201 compounds from the primary HTS with XC_{50} values < 10 μM in the 5-FUrd assay that were extensively characterized.

GSK Blinded Identifier	Plate	Well	IC50 48 hr 3D7 parasite viability (M)	IC50 48hr PfENT1-KO parasite viability (M)	IC50 for inhibition of adenosine-dependent growth of ade2Δ + PfENT1-expressing yeast (M)	IC50 for inhibition of 50 nM [3H]adenosine uptake into ade2Δ + PfENT1-expressing yeast, 15-min uptake assay (M)	IC50 for inhibition of 250 nM [3H]Uridine into ade2Δ + PfENT1-expressing yeast, 15-min uptake assay (M)	HepG2 pIC50 MEAN MOD		HepG2 pIC50 MEAN	HepG2 pIC50 N_INC_IN_MEAN	HepG2 pIC50 N_TOTAL. May be greater than value in column L
			N=3	N=2	N=1	N=2	N=1					
U000H91_A1	**H91**	A1	2.00E-05	2.21E-05	2.41E-06	9.23E-08	7.51E-08	<		4	2	2
U000H91_A2	**H91**	A2	4.55E-05	NE	3.87E-07	3.41E-08	2.79E-08	<		4	2	2
U000H91_A3	**H91**	A3	1.54E-05	2.94E-05	5.09E-07	5.40E-08	3.10E-08	=		4.1	1	2
U000H91_A4	**H91**	A4	NE	NE	1.36E-06	4.41E-08	4.11E-08	<		4	4	4
U000H91_A5	**H91**	A5	7.31E-06	2.24E-05	3.41E-08	2.82E-08	2.73E-08	=		4.2	4	4
U000H91_A6	**H91**	A6	9.84E-06	1.15E-05	1.44E-07	3.30E-06	2.71E-06	<		4	2	2
U000H91_A7	**H91**	A7	NE	NE	2.60E-07	2.63E-07	5.22E-07	<		4	2	2
U000H91_A8	**H91**	A8	5.44E-05	8.02E-05	2.51E-07	9.30E-08	8.43E-08	<		4	2	2
U000H91_A9	**H91**	A9	2.83E-05	low activity	4.53E-07	2.93E-08	3.19E-08	<		4	2	2
U000H91_A10	**H91**	A10	2.56E-05	1.65E-05	2.95E-07	4.50E-08	3.01E-08	<		4	2	2
U000H92_A1	**H92**	A1	4.38E-06	3.70E-06	6.36E-07	3.65E-08	4.63E-08	<		4	2	2
U000H92_A2	**H92**	A2	5.10E-05	4.72E-05	9.52E-07	NE	NE	<		4	2	2
U000H92_A3	**H92**	A3	3.24E-05	3.02E-05	1.47E-06	1.49E-07	4.04E-07	=		4	1	2
U000H92_A4	**H92**	A4	1.72E-05	NE	7.64E-07	NE	NE	<		4	2	2
U000H92_A5	**H92**	A5	5.37E-05	NE	4.05E-07	3.20E-06	4.57E-06	=		4.4	2	2
U000H92_A6	**H92**	A6	4.41E-05	NE	3.48E-07	6.72E-07	6.91E-07	<		4	2	2
U000H92_A7	**H92**	A7	5.30E-06	4.95E-06	2.68E-07	4.26E-08	3.50E-08	<		4	2	2
U000H92_A8	**H92**	A8	6.04E-06	4.00E-05	1.74E-07	4.83E-08	5.93E-08	<		4	2	2
U000H92_A9	**H92**	A9	2.04E-06	1.91E-06	4.85E-07	3.56E-06	3.69E-06	=		4.6	2	2
U000H92_A10	**H92**	A10	1.45E-05	1.65E-05	6.18E-07	3.72E-08	2.89E-08	<		4	2	2
U000H93_A1	**H93**	A1	9.61E-06	1.37E-05	6.63E-07	3.32E-08	3.63E-08	<		4	4	4
U000H93_A2	**H93**	A2	4.54E-05	NE	1.10E-07	2.12E-06	8.76E-07	=		4.1	1	2
U000H93_A3	**H93**	A3	1.11E-05	8.41E-06	5.27E-07	1.41E-07	9.76E-08	<		4	2	2
U000H94_A1	**H94**	A1	3.30E-05	5.12E-05	1.59E-06	6.91E-08	1.25E-07	<		4	2	2
U000H94_A2	**H94**	A2	1.17E-05	1.28E-05	9.65E-07	3.48E-08	4.72E-08	<		4	2	2
U000H94_A3	**H94**	A3	9.60E-06	2.32E-05	2.60E-07	3.42E-08	3.45E-08	=		4.1	2	2
U000H94_A4	**H94**	A4	7.15E-06	6.37E-06	7.47E-07	4.25E-08	6.80E-08	=		4.2	1	2
U000H94_A5	**H94**	A5	2.81E-06	9.39E-06	2.94E-07	3.34E-08	5.60E-08	=		4.3	1	2
U000H94_A6	**H94**	A6	NE	NE	4.45E-07	3.32E-08	3.46E-08	<		4	2	2
U000H94_A7	**H94**	A7	2.65E-05	3.00E-05	3.68E-08	1.54E-06	2.54E-06	=		4.5	1	2
U000H94_A8	**H94**	A8	4.77E-05	6.04E-05	1.45E-06	8.12E-08	1.56E-07	=		4	1	2
U000H94_A9	**H94**	A9	3.27E-05	3.44E-05	4.78E-07	1.95E-06	2.52E-06	=		4.6	4	4
U000H94_A10	**H94**	A10	4.79E-05	4.92E-05	2.33E-07	1.61E-07	2.96E-07	<		4	2	2
U000H97_A1	**H97**	A1	2.56E-05	2.62E-05	1.13E-06	1.40E-06	2.86E-06	<		4	2	2
U000H97_A2	**H97**	A2	2.34E-05	3.23E-05	3.01E-07	7.06E-08	4.45E-08	=		5.1	2	4
U000H97_A3	**H97**	A3	NE	NE	1.16E-07	5.16E-07	7.66E-07	<		4	2	2
U000H97_A4	**H97**	A4	1.62E-05	9.64E-05	3.73E-07	2.83E-07	8.32E-07	<		4	2	2
U000H97_A5	**H97**	A5	3.11E-06	5.77E-06	6.94E-08	4.43E-08	9.56E-08	<		4	4	4
U000H97_A6	**H97**	A6	1.80E-05	1.47E-05	1.19E-08	1.68E-06	3.82E-06	<		4	2	2
U000H97_A7	**H97**	A7	2.72E-05	3.52E-05	4.56E-07	3.51E-08	7.12E-08	<		4	2	2
U000H97_A8	**H97**	A8	5.13E-05	NE	8.25E-07	3.92E-08	5.24E-08	<		4	2	2
U000H97_A9	**H97**	A9	4.24E-05	3.93E-05	4.55E-07	NE	NE	<		4	2	2
U000H97_A10	**H97**	A10	7.25E-05	7.72E-05	1.23E-07	3.33E-06	3.06E-06	<		4	2	2
U000H98_A1	**H98**	A1	2.26E-05	2.81E-05	6.76E-08	9.33E-06	9.44E-06	<		4	2	2
U000H98_A2	**H98**	A2	3.17E-05	4.52E-05	5.12E-07	4.25E-08	5.58E-08	<		4	2	2
U000H98_A3	**H98**	A3	2.13E-06	2.23E-06	3.37E-07	2.54E-07	6.51E-07	<		4	4	4
U000H98_A4	**H98**	A4	1.70E-06	1.09E-05	2.35E-07	6.32E-08	1.02E-07	=		4.3	2	2
U000H98_A5	**H98**	A5	8.48E-06	2.03E-05	7.14E-07	8.52E-08	1.23E-07	<		4	2	2
U000H98_A6	**H98**	A6	3.09E-05	3.22E-05	8.59E-08	1.23E-06	2.84E-06	=		4.4	2	2
U000H98_A7	**H98**	A7	3.16E-05	2.75E-05	4.50E-08	1.39E-07	4.10E-07	<		4	2	2
U000H98_A8	**H98**	A8	2.56E-05	NE	2.03E-07	3.81E-08	4.57E-08	=		4.2	2	2
U000H98_A9	**H98**	A9	3.09E-05	2.66E-05	1.46E-06	4.14E-06	5.54E-06	<		4	2	2
U000H98_A10	**H98**	A10	2.92E-05	2.90E-05	5.87E-06	6.89E-07	1.14E-06	=		4.4	2	2
U000H99_A1	**H99**	A1	4.13E-05	NE	9.75E-07	6.83E-08	6.35E-08	<		4	2	2

U000H99_A2	**H99**	A2	2.97E-05	3.59E-04	6.67E-07	2.89E-06	3.80E-06	=	4.2	2	2
U000H99_A3	**H99**	A3	NE	NE	3.26E-08	1.44E-05	low activity	<	4	2	2
U000H99_A4	**H99**	A4	3.12E-05	3.08E-05	3.27E-07	4.19E-06	4.90E-07	=	4.3	2	2
U000H99_A5	**H99**	A5	3.94E-05	NE	1.30E-07	6.84E-08	3.90E-08	<	4	2	2
U000H99_A6	**H99**	A6	6.61E-05	low activity	5.97E-08	7.52E-08	1.60E-07	<	4	2	2
U000H99_A7	**H99**	A7	3.44E-05	8.33E-05	3.57E-07	6.17E-06	low activity	=	4	1	2
U000H99_A8	**H99**	A8	4.37E-05	NE	3.66E-07	1.04E-05	low activity	<	4	2	2
U000H99_A9	**H99**	A9	9.62E-05	NE	1.81E-07	2.38E-06	6.71E-06	=	4	1	2
U000H99_A10	**H99**	A10	NE	NE	3.75E-07	2.19E-07	7.50E-07	<	4	2	2
U000H9A_A1	**H9A**	A1	1.27E-05	4.83E-05	2.84E-07	2.86E-08	3.51E-08	<	4	2	2
U000H9A_A2	**H9A**	A2	1.80E-05	ND	ND	3.19E-08	3.02E-08	<	4	2	2
U000H9A_A3	**H9A**	A3	4.17E-05	NE	7.22E-07	NE	NE	=	4.1	1	2
U000H9A_A4	**H9A**	A4	NE	low activity	NE	NE	NE	<	4	2	2
U000H9A_A5	**H9A**	A5	3.32E-05	9.30E-05	6.81E-07	1.98E-06	low activity	<	4	2	2
U000H9A_A6	**H9A**	A6	8.94E-05	9.75E-05	2.03E-06	3.20E-06	5.55E-06	<	4	2	2
U000H9A_A7	**H9A**	A7	2.00E-05	5.31E-05	1.87E-06	1.90E-06	4.13E-06	<	4	2	2
U000H9A_A8	**H9A**	A8	3.09E-05	4.72E-05	4.24E-07	1.56E-06	3.92E-06	<	4	2	2
U000H9A_A9	**H9A**	A9	1.81E-05	3.80E-05	5.47E-07	6.53E-08	3.80E-08	<	4	2	2
U000H9A_A10	**H9A**	A10	3.08E-05	4.93E-05	6.41E-07	7.08E-06	low activity	<	4	4	4
U000H9B_A1	**H9B**	A1	3.35E-05	3.81E-05	2.19E-06	low activity	low activity	=	4.3	2	2
U000H9B_A2	**H9B**	A2	2.86E-05	5.13E-05	3.08E-07	4.97E-07	9.83E-07	=	4.1	2	2
U000H9B_A3	**H9B**	A3	1.37E-05	1.26E-05	1.17E-06	6.29E-06	1.77E-06	=	4.4	1	2
U000H9B_A4	**H9B**	A4	9.95E-05	NE	3.65E-07	NE	NE	<	4	2	2
U000H9B_A5	**H9B**	A5	low activity	NE	2.14E-07	3.23E-08	8.30E-08	<	4	2	2
U000H9B_A6	**H9B**	A6	3.45E-05	low activity	2.27E-07	low activity	low activity	<	4	2	2
U000H9B_A7	**H9B**	A7	3.24E-05	low activity	8.40E-07	2.96E-08	9.50E-08	<	4	2	2
U000H9B_A8	**H9B**	A8	3.04E-05	4.66E-05	5.23E-07	low activity	NE	=	4.2	2	2
U000H9B_A9	**H9B**	A9	1.75E-05	1.85E-05	2.51E-07	2.93E-08	9.98E-08	=	4.3	2	2
U000H9B_A10	**H9B**	A10	2.78E-05	7.09E-05	1.10E-07	4.08E-08	9.97E-08	<	4	2	2
U000H9D_A1	**H9D**	A1	3.50E-05	2.71E-05	8.87E-07	4.15E-08	4.31E-08	<	4	2	2
U000H9D_A2	**H9D**	A2	NE	NE	3.67E-07	5.02E-08	8.00E-08	ND	ND	ND	ND
U000H9D_A3	**H9D**	A3	3.04E-05	2.80E-05	3.62E-08	2.72E-06	8.58E-06	=	4.6	2	2
U000H9D_A4	**H9D**	A4	1.03E-05	NE	1.23E-06	3.98E-06	6.13E-06	<	4	2	2
U000H9D_A5	**H9D**	A5	3.63E-05	6.59E-05	1.32E-06	1.14E-06	2.70E-06	=	4.3	2	2
U000H9D_A6	**H9D**	A6	7.98E-06	6.23E-06	1.11E-06	low activity	low activity	=	4.6	1	2
U000H9D_A7	**H9D**	A7	3.29E-05	3.32E-05	1.50E-06	7.99E-07	2.42E-06	=	4.3	2	2
U000H9D_A8	**H9D**	A8	1.92E-05	NE	4.67E-07	4.41E-08	5.34E-08	<	4	2	2
U000H9D_A9	**H9D**	A9	1.67E-05	1.96E-05	1.06E-06	4.46E-08	8.46E-08	=	4.1	2	2
U000H9D_A10	**H9D**	A10	7.98E-06	1.43E-05	5.93E-07	7.02E-08	9.06E-08	=	4.3	2	2
U000H9E_A1	**H9E**	A1	7.89E-07	2.42E-05	5.37E-07	1.23E-07	4.50E-07	<	4	2	2
U000H9E_A2	**H9E**	A2	NE	low activity	5.39E-07	4.43E-08	3.48E-08	<	4	2	2
U000H9E_A3	**H9E**	A3	5.95E-05	low activity	1.34E-07	7.81E-07	4.02E-06	<	4	2	2
U000H9E_A4	**H9E**	A4	NE	1.70E-05	ND	1.14E-07	4.05E-07	<	4	2	2
U000H9E_A5	**H9E**	A5	1.41E-05	2.65E-05	4.50E-07	1.16E-07	4.96E-08	=	4	1	2
U000H9E_A6	**H9E**	A6	NE	NE	4.16E-07	4.32E-06	1.07E-05	<	4	2	2
U000H9E_A7	**H9E**	A7	1.17E-05	3.93E-06	4.45E-07	4.13E-08	4.21E-08	=	4.4	2	2
U000H9E_A8	**H9E**	A8	3.38E-05	3.61E-05	2.02E-06	1.69E-06	6.85E-06	=	4.3	2	2
U000HAV_A1	**HAV**	A1	1.52E-05	4.17E-05	1.79E-07	1.29E-06	9.21E-07	<	4	2	2
U000HAV_A2	**HAV**	A2	3.55E-05	3.63E-05	8.29E-07	8.59E-06	3.34E-06	<	4	2	2
U000HAV_A3	**HAV**	A3	3.06E-05	3.68E-05	6.51E-07	4.62E-08	3.93E-08	=	4.4	1	2
U000HAV_A4	**HAV**	A4	3.14E-05	3.73E-05	4.14E-07	2.17E-06	3.36E-06	=	4.4	3	3
U000HAV_A5	**HAV**	A5	4.60E-05	5.99E-05	4.22E-06	7.06E-06	8.73E-06	=	4.5	1	2
U000HAV_A6	**HAV**	A6	4.89E-05	5.59E-05	9.67E-08	3.40E-06	3.51E-06	=	4.4	1	2
U000HAV_A7	**HAV**	A7	1.47E-05	3.27E-05	7.13E-08	2.79E-08	2.85E-08	=	4.5	1	2
U000HAV_A8	**HAV**	A8	4.75E-05	6.69E-05	5.88E-07	3.67E-06	4.89E-06	=	4.2	2	2
U000HAV_A9	**HAV**	A9	1.69E-05	2.11E-05	2.70E-07	5.16E-06	1.02E-05	<	4	4	4
U000HAV_A10	**HAV**	A10	1.77E-05	2.26E-05	5.04E-07	4.43E-08	5.63E-08	=	4.6	1	2
U000HT5_A1	**HT5**	A1	1.28E-04	3.19E-05	2.99E-07	9.01E-08	2.02E-07	<	4	2	2
U000HT5_A2	**HT5**	A2	1.00E-05	1.86E-05	5.97E-08	5.52E-07	1.19E-06	<	4	2	2
U000HT5_A3	**HT5**	A3	NE	low activity	NE	3.25E-06	9.50E-06	<	4	2	2
U000HT5_A4	**HT5**	A4	2.17E-06	7.11E-06	1.46E-07	5.48E-08	4.43E-08	<	4	4	4
U000HT5_A5	**HT5**	A5	1.70E-05	2.34E-05	3.53E-07	2.14E-07	2.88E-07	<	4	2	2
U000HT5_A6	**HT5**	A6	2.66E-05	4.02E-05	4.15E-07	1.27E-07	1.35E-07	<	4	2	2

U000HT5_A7	**HT5**	A7	NE	low activity	8.98E-07	1.13E-07	5.22E-07	<	4	2	2
U000HT5_A8	**HT5**	A8	3.03E-05	4.20E-05	1.59E-06	5.13E-08	5.29E-08	<	4	2	2
U000HT5_A9	**HT5**	A9	2.97E-05	2.74E-05	5.41E-07	3.40E-06	5.80E-06	=	4.3	2	2
U000HT5_A10	**HT5**	A10	2.36E-05	1.25E-05	5.03E-07	3.69E-08	3.84E-08	<	4	2	2
U000HT6_A1	**HT6**	A1	2.25E-06	2.64E-06	1.18E-07	3.44E-08	6.04E-08	<	4	4	4
U000HT6_A2	**HT6**	A2	6.88E-06	3.87E-05	4.33E-07	1.95E-08	3.43E-08	<	4	2	2
U000HT6_A3	**HT6**	A3	NE	8.88E-05	9.99E-07	2.06E-06	2.84E-06	<	4	2	2
U000HT6_A4	**HT6**	A4	8.73E-06	3.62E-05	1.81E-07	4.70E-08	3.68E-08	<	4	2	2
U000HT6_A5	**HT6**	A5	3.98E-05	4.61E-05	4.11E-08	2.94E-07	3.01E-07	<	4	2	2
U000HT6_A6	**HT6**	A6	2.47E-05	7.35E-05	2.41E-07	3.54E-08	3.04E-08	<	4	2	2
U000HT6_A7	**HT6**	A7	NE	9.74E-05	7.60E-07	4.99E-07	1.11E-06	<	4	2	2
U000HT6_A8	**HT6**	A8	2.90E-06	1.96E-05	1.46E-07	3.57E-08	2.98E-08	<	4	2	2
U000HT6_A9	**HT6**	A9	1.83E-05	1.65E-05	4.88E-07	3.67E-06	5.23E-06	=	4.3	4	4
U000HT6_A10	**HT6**	A10	1.28E-05	1.65E-05	2.09E-07	4.77E-08	5.14E-08	=	4.2	2	2
U000HT7_A1	**HT7**	A1	1.29E-04	NE	1.37E-06	3.77E-08	7.50E-08	<	4	2	2
U000HT7_A2	**HT7**	A2	low activity	4.63E-05	9.99E-07	4.69E-08	5.55E-08	=	4.2	1	2
U000HT7_A3	**HT7**	A3	NE	NE	8.25E-07	3.68E-08	3.81E-08	<	4	2	2
U000HT7_A4	**HT7**	A4	NE	NE	4.49E-07	6.58E-08	4.48E-08	<	4	2	2
U000HT7_A5	**HT7**	A5	5.21E-05	2.34E-05	5.80E-07	7.50E-08	2.90E-08	<	4	1	1
U000HT7_A6	**HT7**	A6	3.79E-06	6.27E-06	2.82E-07	5.82E-08	3.57E-08	<	4	2	2
U000HT7_A7	**HT7**	A7	3.26E-05	4.39E-05	5.73E-07	8.35E-07	5.52E-06	=	4.3	2	2
U000HT7_A8	**HT7**	A8	7.06E-05	NE	5.32E-07	2.99E-06	4.83E-06	<	4	2	2
U000HT7_A9	**HT7**	A9	1.21E-05	1.84E-05	3.20E-07	5.34E-08	3.11E-08	ND	ND	ND	ND
U000HT7_A10	**HT7**	A10	3.14E-05	2.33E-05	2.08E-06	4.71E-07	7.47E-07	=	4.3	2	2
U000HT8_A1	**HT8**	A1	1.79E-05	4.18E-05	2.61E-07	4.93E-08	7.32E-08	<	4	2	2
U000HT8_A2	**HT8**	A2	5.27E-05	2.56E-05	1.31E-07	2.64E-06	2.30E-06	=	4.4	2	2
U000HT8_A3	**HT8**	A3	3.44E-05	5.51E-05	2.14E-07	4.77E-08	4.54E-08	<	4	2	2
U000HT8_A4	**HT8**	A4	NE	low activity	1.43E-07	9.17E-08	1.96E-07	<	4	2	2
U000HT8_A5	**HT8**	A5	NE	low activity	2.53E-08	5.07E-06	3.97E-06	<	4	2	2
U000HT8_A6	**HT8**	A6	3.24E-05	9.29E-06	4.00E-07	3.56E-06	7.02E-06	=	4.8	1	2
U000HT8_A7	**HT8**	A7	1.12E-05	2.06E-05	1.79E-07	1.31E-06	2.01E-06	<	4	2	2
U000HT8_A8	**HT8**	A8	2.25E-05	2.58E-05	1.08E-06	5.03E-08	5.04E-08	<	4	2	2
U000HT8_A9	**HT8**	A9	2.00E-05	2.57E-05	3.68E-07	1.03E-06	1.44E-06	=	4.7	2	2
U000HT8_A10	**HT8**	A10	8.65E-06	6.66E-05	3.24E-07	4.80E-08	6.03E-08	<	4	2	2
U000HTR_A1	**HTR**	A1	2.38E-05	2.65E-05	5.16E-07	1.85E-07	3.12E-07	<	4	2	2
U000HTR_A2	**HTR**	A2	NE	NE	3.12E-07	3.83E-07	4.24E-07	<	4	2	2
U000HTR_A3	**HTR**	A3	7.20E-05	low activity	2.33E-06	4.69E-08	1.06E-07	<	4	6	6
U000HTR_A4	**HTR**	A4	2.95E-05	2.59E-05	2.31E-07	low activity	low activity	<	4	2	2
U000HTR_A5	**HTR**	A5	NE	1.40E-05	2.75E-07	low activity	low activity	<	4	2	2
U000HTR_A6	**HTR**	A6	2.91E-05	2.07E-05	5.30E-07	3.44E-06	1.36E-06	<	4	4	4
U000HTR_A7	**HTR**	A7	8.55E-06	6.92E-06	8.81E-07	low activity	low activity	=	5	1	2
U000HTR_A8	**HTR**	A8	4.12E-06	6.17E-06	1.60E-07	4.11E-08	6.00E-08	<	4	2	2
U000HTR_A9	**HTR**	A9	3.01E-05	2.92E-05	1.94E-07	2.30E-06	2.75E-06	=	4.6	2	2
U000HTR_A10	**HTR**	A10	4.00E-05	2.01E-05	5.71E-07	3.58E-06	1.75E-06	=	4.1	1	2
U000HTU_A1	**HTU**	A1	1.59E-05	2.10E-05	2.26E-07	3.85E-08	7.73E-08	=	4.3	1	2
U000HTU_A2	**HTU**	A2	6.37E-06	low activity	1.67E-07	2.93E-08	3.39E-08	<	4	2	2
U000HTU_A3	**HTU**	A3	NE	NE	4.92E-07	3.01E-08	4.29E-08	<	4	2	2
U000HTU_A4	**HTU**	A4	5.00E-05	2.00E-05	2.24E-07	4.30E-07	6.22E-07	<	4	2	2
U000HTU_A5	**HTU**	A5	3.59E-05	3.67E-05	1.40E-07	5.18E-08	1.09E-07	<	4	4	4
U000HTU_A6	**HTU**	A6	2.52E-05	1.52E-05	1.14E-07	6.60E-08	1.04E-07	=	4.2	1	2
U000HTU_A7	**HTU**	A7	7.50E-06	1.14E-05	2.01E-07	7.13E-07	1.25E-06	=	4.4	2	2
U000HTU_A8	**HTU**	A8	4.57E-05	9.85E-05	1.78E-07	2.43E-07	6.87E-07	=	4.1	2	2
U000HTU_A9	**HTU**	A9	1.86E-04	NE	2.43E-07	3.14E-08	3.21E-08	<	4	2	2
U000HTU_A10	**HTU**	A10	3.16E-05	3.00E-05	3.29E-07	9.07E-07	1.88E-06	=	4.3	2	2
U000HTV_A1	**HTV**	A1	8.08E-06	6.04E-06	2.54E-06	2.36E-06	2.28E-06	=	4.3	2	2
U000HTV_A2	**HTV**	A2	1.39E-05	1.08E-05	2.06E-07	3.99E-07	6.08E-07	<	4	4	4
U000HTV_A3	**HTV**	A3	1.34E-05	1.45E-05	1.93E-07	1.22E-06	2.08E-06	ND	ND	ND	ND
U000HTV_A4	**HTV**	A4	1.05E-05	2.92E-05	6.38E-07	4.52E-08	5.43E-08	<	3.9	2	2
U000HTV_A5	**HTV**	A5	NE	NE	1.38E-07	NE	NE	<	4	2	2
U000HTV_A6	**HTV**	A6	3.71E-05	3.52E-05	6.63E-07	2.46E-06	1.70E-06	=	4.3	1	2
U000HTV_A7	**HTV**	A7	2.20E-05	1.48E-05	1.79E-06	6.76E-08	8.55E-08	<	4	2	2
U000HTV_A8	**HTV**	A8	5.09E-05	3.73E-05	7.42E-07	4.96E-06	6.23E-06	=	4.4	1	2
U000HTV_A9	**HTV**	A9	low activity	NE	5.46E-07	1.56E-06	2.75E-06	=	4	1	2

U000HTV_A10	**HTV**	A10	1.73E-05	NE	3.23E-07	3.02E-08	2.84E-08	<	4	2	2
U000HTW_A1	**HTW**	A1	2.68E-05	3.21E-05	1.37E-06	2.32E-06	3.69E-06	<	4	2	2
U000HTW_A2	**HTW**	A2	2.78E-05	1.83E-05	9.99E-07	5.74E-07	9.91E-07	<	4	2	2
U000HTW_A3	**HTW**	A3	4.64E-06	2.91E-06	8.25E-07	1.74E-07	2.32E-07	=	4.6	1	2
U000HTW_A4	**HTW**	A4	8.74E-06	7.78E-06	4.49E-07	1.86E-06	2.06E-06	=	4.4	1	2
U000HTW_A5	**HTW**	A5	1.75E-05	1.78E-05	5.80E-07	1.55E-06	2.08E-06	<	4	4	4
U000HTW_A6	**HTW**	A6	4.28E-05	4.48E-05	2.82E-07	2.21E-06	4.36E-06	<	4	2	2
U000HTW_A7	**HTW**	A7	2.17E-05	1.48E-05	5.73E-07	5.91E-08	8.41E-08	<	4	2	2
U000HTW_A8	**HTW**	A8	2.79E-05	2.45E-05	5.32E-07	2.54E-06	6.64E-06	=	4.5	2	2
U000HTW_A9	**HTW**	A9	3.51E-05	3.79E-05	3.20E-07	3.70E-08	8.11E-08	=	4.1	3	6
U000HTW_A10	**HTW**	A10	1.87E-05	5.32E-05	2.08E-06	3.02E-08	3.98E-08	=	4.2	1	2
U000HU7_A1	**HU7**	A1	3.81E-05	low activity	2.57E-07	3.45E-08	2.90E-08	<	4	2	2
U000HU7_A2	**HU7**	A2	4.63E-05	7.51E-05	7.49E-08	5.74E-06	4.15E-06	<	4	2	2
U000HU7_A3	**HU7**	A3	4.33E-06	1.76E-05	8.38E-08	3.59E-08	3.25E-08	=	4.4	2	2
U000HU7_A4	**HU7**	A4	2.21E-05	low activity	2.19E-07	4.30E-08	4.08E-08	=	4.1	1	2
U000HU7_A5	**HU7**	A5	8.15E-05	low activity	3.26E-07	5.73E-08	3.84E-08	<	4	2	2
U000HU7_A6	**HU7**	A6	6.15E-05	low activity	2.24E-07	6.53E-08	2.83E-08	<	4	2	2
U000HU7_A7	**HU7**	A7	3.27E-05	3.47E-05	3.73E-07	5.31E-08	4.69E-08	<	4	2	2
U000HU7_A8	**HU7**	A8	NE	NE	1.86E-06	6.13E-08	6.43E-08	<	4	2	2
U000HU7_A9	**HU7**	A9	3.05E-05	low activity	2.57E-07	5.05E-08	6.19E-08	<	4	2	2
U000HU7_A10	**HU7**	A10	2.38E-06	low activity	4.61E-07	3.47E-08	3.86E-08	<	4	2	2

NOTES

NE, no effect up to maximum concentration tested

Low activity, effect at highest concentration tested, but insufficient data to calculate IC50 value

ND, not determined

HepG2 data were extracted fresh from corporate database on 6/15/18.

Supplementary Table S3. XC_{50} values for various assays performed on 123 compounds (Wave 2) that were identified by GSK chemists during hit expansion.

GSK Blinded Identifier	IC50 48 hr 3D7 parasite viability (M)	IC50 48hr PfENT1-KO parasite viability (M)	IC50 for inhibition of adenosine-dependent growth of ade2Δ + PfENT1-expressing yeast (M)	IC50 for inhibition of 50 nM [3H]adenosine uptake into ade2Δ + PfENT1-expressing yeast, 15-min uptake assay (M)	IC50 for inhibition of 250 nM [3H]Uridine into ade2Δ + PfENT1-expressing yeast, 15-min uptake assay (M)
	N=3	N=2	N=1	N=1	N=1
U001RV1_A1	8.88E-06	1.08E-05	5.32E-06	2.59E-06	6.16E-06
U001RV1_A2	1.19E-05	3.87E-05	5.80E-07	1.91E-07	1.29E-07
U001RV1_A3	2.24E-05	3.25E-05	4.99E-07	1.29E-07	6.27E-08
U001RV1_A4	2.82E-05	1.02E-04	1.53E-06	1.61E-07	1.33E-07
U001RV1_A5	1.77E-05	6.26E-05	6.11E-07	8.63E-08	6.15E-08
U001RV1_A6	1.23E-05	1.45E-05	4.05E-06	1.24E-06	1.31E-06
U001RV1_A7	2.04E-05	4.61E-05	6.88E-07	8.35E-08	6.74E-08
U001RV1_A8	1.75E-05	NE	3.31E-05	2.63E-06	5.03E-06
U001RV1_A9	2.18E-05	3.52E-05	1.03E-05	1.04E-05	2.89E-05
U001RV1_A10	2.83E-06	NE	2.26E-05	1.09E-06	2.10E-06
U001RV2_A1	2.83E-05	8.40E-05	1.16E-06	1.58E-07	6.45E-08
U001RV2_A2	2.03E-05	6.17E-05	1.22E-06	1.30E-06	3.40E-06
U001RV2_A3	NE	1.61E-03	1.40E-06	1.74E-05	2.52E-05
U001RV2_A4	3.19E-05	5.49E-05	9.23E-07	2.20E-07	2.77E-07
U001RV2_A5	NE	low activity	1.82E-06	2.42E-05	2.44E-05
U001RV2_A6	2.51E-05	7.53E-05	3.97E-07	1.33E-07	1.23E-07
U001RV2_A7	NE	low activity	5.06E-08	6.48E-07	1.49E-06
U001RV2_A8	NE	low activity	1.04E-06	6.43E-06	1.21E-05
U001RV2_A9	6.27E-06	6.78E-06	9.41E-07	1.41E-07	1.32E-07
U001RV2_A10	1.92E-05	2.78E-04	NE	NE	NE
U001RXG_A1	1.51E-04	4.19E-03	6.09E-06	2.18E-06	3.15E-06
U001RXG_A2	5.96E-05	6.90E-05	7.67E-07	1.43E-07	4.13E-08
U001RXG_A3	5.71E-05	6.60E-05	2.01E-06	1.48E-07	8.92E-08
U001RXG_A4	1.84E-05	5.50E-05	1.80E-06	1.26E-07	6.13E-08
U001RXG_A5	3.38E-05	3.06E-05	5.51E-08	8.09E-08	1.43E-08
U001RXG_A6	NE	NE	1.78E-06	2.30E-06	2.94E-06
U001RXG_A7	NE	NE	8.93E-06	3.50E-06	7.01E-06
U001RXG_A8	NE	NE	1.96E-06	1.08E-07	4.47E-08
U001RXG_A9	3.76E-05	7.11E-05	3.47E-06	NE	NE
U001RXG_A10	2.95E-05	6.61E-05	2.76E-06	NE	NE
U001RXH_A1	NE	NE	1.82E-06	7.71E-06	1.76E-05
U001RXH_A2	NE	NE	4.31E-06	3.85E-06	5.37E-06
U001RXH_A3	2.69E-05	1.57E-02	4.46E-06	1.68E-07	3.22E-07
U001RXH_A4	NE	NE	6.40E-06	9.54E-08	1.21E-07
U001RXH_A5	3.06E-05	3.99E-05	2.91E-06	NE	NE
U001RXH_A6	8.10E-06	9.31E-06	6.83E-07	NE	NE
U001RXH_A7	4.57E-05	2.10E-04	5.01E-06	NE	NE
U001RXH_A8	4.79E-05	NE	6.12E-06	3.95E-07	6.43E-07
U001RXH_A9	NE	3.12E-05	2.02E-05	1.26E-06	1.38E-06

U001RXH_A10	4.36E-05	6.59E-05	1.71E-06	1.77E-07	2.29E-07
U001RXJ_A1	4.33E-05	7.16E-05	2.17E-06	2.17E-07	3.80E-07
U001RXJ_A2	4.12E-05	4.68E-05	1.40E-06	2.05E-05	1.26E-05
U001RXJ_A3	3.26E-05	low activity	1.53E-06	1.81E-07	2.73E-07
U001RXJ_A4	7.26E-05	NE	3.42E-07	1.19E-07	1.11E-07
U001RXJ_A5	low activity	low activity	7.21E-06	NE	NE
U001RXJ_A6	1.61E-05	1.93E-05	9.74E-07	9.29E-06	1.38E-05
U001RXJ_A7	4.14E-05	5.04E-05	2.35E-06	2.50E-07	3.55E-07
U001RXJ_A8	7.91E-06	1.12E-05	1.11E-06	7.15E-07	6.08E-07
U001RXJ_A9	NE	NE	1.13E-05	NE	NE
U001RXJ_A10	NE	low activity	2.09E-05	NE	NE
U001RXK_A1	3.50E-05	1.08E-04	4.77E-06	2.45E-05	2.74E-05
U001RXK_A2	2.87E-05	5.82E-05	1.26E-06	1.95E-07	3.03E-07
U001RXK_A3	3.21E-05	8.97E-05	8.86E-07	1.10E-07	1.41E-07
U001RXK_A4	3.33E-05	1.05E-04	1.24E-06	1.08E-07	1.58E-07
U001RXK_A5	5.14E-04	NE	9.14E-07	1.65E-07	1.61E-07
U001RXK_A6	1.43E-05	2.48E-04	4.06E-07	8.39E-08	9.44E-08
U001RXK_A7	9.97E-06	2.09E-05	7.89E-07	4.39E-07	5.25E-07
U001RXK_A8	low activity	4.63E-05	1.23E-06	8.21E-06	9.86E-06
U001RXK_A9	NE	NE	2.69E-06	2.31E-07	4.36E-07
U001RXK_A10	4.60E-05	1.02E-04	1.80E-06	1.57E-07	2.09E-07
U001RUY_A1	2.66E-05	7.50E-05	6.95E-06	2.20E-07	1.50E-07
U001RUY_A2	2.59E-05	1.27E-04	1.73E-06	NE	NE
U001RUY_A3	2.78E-05	1.71E-04	1.56E-06	NE	NE
U001RUY_A4	2.85E-05	NE	4.49E-06	1.44E-05	2.19E-05
U001RUY_A5	low activity	low activity	8.47E-07	1.73E-05	2.02E-05
U001RUY_A6	3.85E-05	low activity	1.54E-06	2.72E-05	2.40E-05
U001RUY_A7	2.71E-05	6.56E-05	1.54E-06	1.96E-06	3.41E-06
U001RUY_A8	1.19E-05	2.71E-05	4.45E-07	2.75E-07	1.71E-07
U001RUY_A9	6.59E-06	2.35E-05	7.43E-07	2.18E-07	1.74E-07
U001RUY_A10	NE	NE	2.25E-06	3.48E-06	9.78E-06
U001RUZ_A1	2.61E-05	3.27E-05	8.96E-07	3.06E-07	2.41E-07
U001RUZ_A2	1.22E-05	low activity	2.93E-07	1.43E-07	1.08E-07
U001RUZ_A3	2.98E-05	1.21E-04	1.79E-06	1.04E-06	4.54E-06
U001RUZ_A4	5.38E-06	NE	1.01E-06	3.22E-06	6.06E-06
U001RUZ_A5	1.63E-05	2.39E-05	6.43E-07	1.69E-07	1.04E-07
U001RUZ_A6	4.62E-06	low activity	2.04E-07	1.80E-05	2.43E-05
U001RUZ_A7	8.64E-06	1.19E-04	7.73E-07	1.26E-07	1.01E-07
U001RUZ_A8	2.14E-05	low activity	1.73E-05	NE	NE
U001RUZ_A9	2.52E-05	7.95E-05	2.41E-06	NE	NE
U001RUZ_A10	low activity	3.48E-05	3.63E-06	1.80E-07	1.53E-07
U001RV0_A1	low activity	low activity	1.05E-06	1.30E-07	7.72E-08
U001RV0_A2	low activity	low activity	1.00E-06	1.39E-07	1.09E-07
U001RV0_A3	3.01E-05	3.45E-05	1.33E-06	2.23E-07	2.53E-07
U001RV0_A4	NE	NE	1.43E-06	2.04E-07	1.65E-07
U001RV0_A5	8.72E-06	8.96E-06	1.00E-06	7.89E-06	8.32E-06
U001RV0_A6	low activity	NE	7.14E-07	2.79E-05	2.75E-05

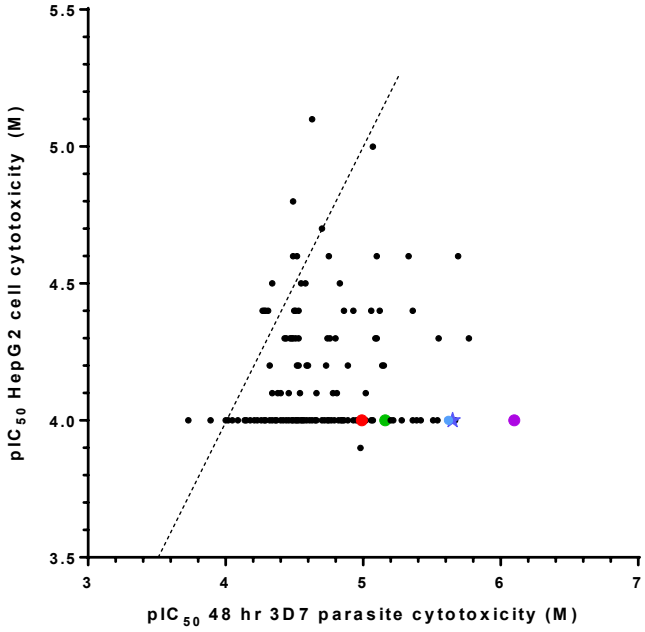
U001RV0_A7	2.23E-05	3.24E-05	2.31E-06	1.12E-07	9.72E-08
U001RV0_A8	1.72E-05	1.68E-05	8.44E-07	3.11E-06	1.32E-05
U001RV0_A9	low activity	3.74E-04	1.36E-06	1.19E-07	8.01E-08
U001RV0_A10	4.06E-05	7.27E-05	2.66E-06	1.40E-07	1.59E-07
U001RXB_A1	NE	NE	2.93E-06	4.40E-06	1.17E-05
U001RXB_A2	3.49E-05	7.03E-05	1.58E-06	2.05E-07	1.72E-07
U001RXB_A3	1.30E-05	NE	9.88E-07	1.60E-07	1.05E-07
U001RXB_A4	1.96E-05	2.41E-05	2.01E-06	2.66E-07	2.92E-07
U001RXB_A5	2.73E-05	4.82E-05	3.51E-06	1.69E-05	2.80E-05
U001RXB_A6	2.62E-05	4.05E-01	2.00E-06	1.55E-07	4.51E-08
U001RXB_A7	NE	NE	2.62E-06	2.17E-06	6.07E-06
U001RXB_A8	2.11E-05	1.35E-04	9.95E-07	1.35E-07	5.83E-08
U001RXB_A9	2.21E-05	8.70E-05	3.89E-06	2.01E-07	2.64E-07
U001RXB_A10	2.71E-05	3.66E-05	7.96E-07	1.66E-07	9.59E-08
U001RXI_A1	NE	NE	1.99E-05	2.73E-05	2.59E-05
U001RXI_A2	NE	NE	2.99E-06	2.61E-06	3.75E-06
U001RXI_A3	NE	NE	1.41E-06	NE	NE
U001RXI_A4	low activity	1.27E-03	4.15E-06	NE	NE
U001RXI_A5	NE	NE	3.38E-06	5.13E-06	7.03E-06
U001RXI_A6	NE	NE	3.55E-06	NE	NE
U001RXI_A7	6.90E-06	2.25E-05	9.52E-07	4.50E-07	4.70E-07
U001RXI_A8	NE	1.06E-04	1.53E-06	1.89E-07	1.93E-07
U001RXI_A9	NE	NE	5.18E-06	NE	NE
U001RXI_A10	4.82E-05	low activity	1.86E-06	NE	NE
U001RXL_A1	NE	NE	NE	NE	NE
U001RXL_A2	6.86E-05	1.40E-04	NE	NE	NE
U001RXL_A3	NE	NE	NE	NE	NE
U001RXU_A1	3.55E-05	1.93E-05	6.33E-06	1.19E-05	1.75E-05
U001RXU_A2	NE	NE	2.88E-06	NE	NE
U001RXU_A3	NE	low activity	4.82E-06	NE	NE
U001RXU_A4	NE	low activity	3.66E-06	9.24E-06	8.60E-06
U001RXU_A5	3.17E-06	9.82E-06	4.07E-07	6.60E-07	3.89E-07
U001RXU_A6	NE	low activity	9.65E-07	NE	NE
U001RXU_A7	1.29E-05	9.66E-06	4.64E-07	1.55E-07	1.39E-07
U001RXU_A8	3.18E-06	9.47E-06	5.26E-08	9.46E-08	7.86E-08
U001RXU_A9	9.09E-06	NE	3.23E-06	1.80E-07	1.98E-07
U001RXU_A10	1.41E-05	2.22E-05	6.01E-07	1.89E-07	2.31E-07

NOTES

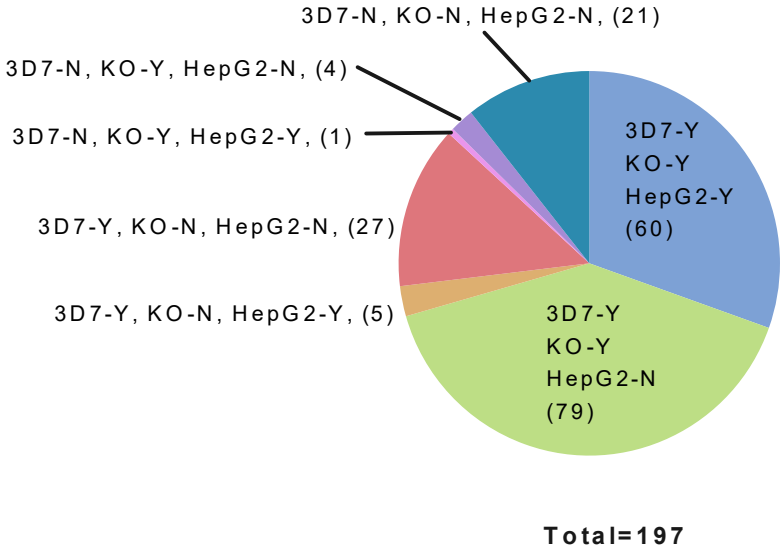
NE, no effect up to maximum concentration tested

Low activity, effect at highest concentration tested, but insufficient data to calculate IC50 value

A

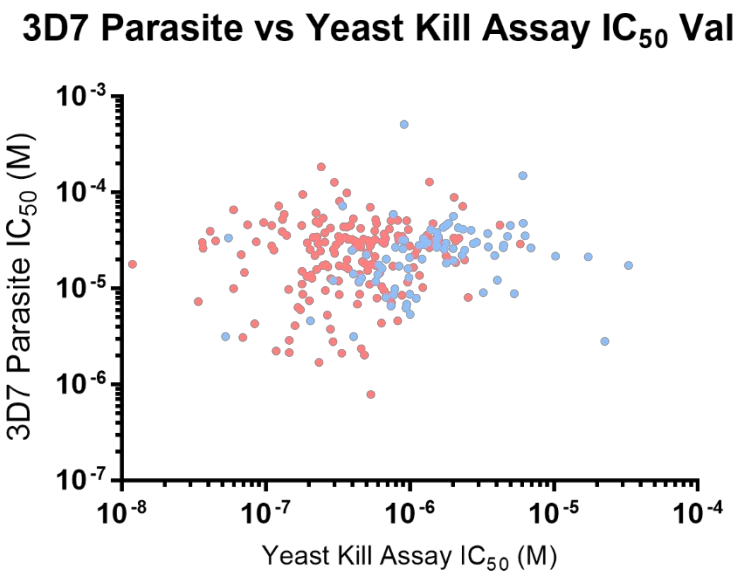


B



Supplementary Figure S1. Comparison of cytotoxicity of the 201 HTS hits for parasites and HepG2 cells. (**A**) For each of the 201 HTS hits, the pIC_{50} value for inhibition human hepatoma HepG2 cell growth (y-axis) is plotted as a function of the pIC_{50} value for inhibition of wild-type 3D7 strain *P. falciparum* parasites proliferation (x-axis). Compounds for which there was no effect at the highest concentration tested in the HepG2 assay, 100 μM, are plotted at pIC_{50} = 4.

The colored symbols identify five of the six extensively characterized compounds GSK-1 (purple), GSK-2 (dark blue), GSK-3 (light blue), GSK-4 (green), GSK-5 (orange), GSK-6 (red). The symbol for GSK-2 is a star to differentiate it from GSK-3. GSK-5 was identified during hit expansion and HepG2 cytotoxicity was not determined. (**B**) Pie chart showing the number of compounds that were (Y) or were not (N) cytotoxic for WT 3D7 parasites, *pfent1*Δ (KO) parasites, and HepG2 cells. The number of compounds in each group is indicated in parentheses. Cytotoxicity was not determined in one of the three assays for four compounds, which are excluded from the analysis.

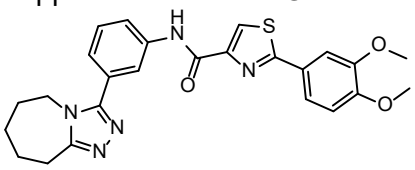


Supplementary Figure S2. Comparison of IC_{50} values in the 3D7 parasite proliferation assay and in the Yeast Kill Assay. Wave 1 compounds, pink symbols. Wave 2 compounds, blue symbols.

Supplemental Data – ^{1}H and ^{13}C NMR data

The structures of all six commercially-available compounds (GSK-1 to GSK-6) were confirmed by ^{1}H and ^{13}C NMR using both 1D and 2D methods. ^{1}H and ^{13}C NMR data were assigned based on 2D COSY, HSQC, and HMBC correlations (see associated data tables).

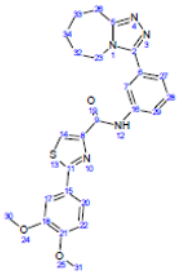
Supplemental Data – GSK-1 NMR spectra



2-(3,4-Dimethoxyphenyl)-N-[3-(6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[4,3-a]azepin-3-yl)phenyl]-1,3-thiazole-4-carboxamide (Enamine cat # Z115045922).

^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 10.43 (s, 1H), 8.43 (s, 1H), 8.11 (s, 1H), 8.01 (d, *J*=8.4 Hz, 1H), 7.73 (d, *J*=1.9 Hz, 1H), 7.66 (d, *J*=8.4 Hz, 1H), 7.56 (t, *J*=7.7 Hz, 1H), 7.31 (d, *J*=7.6 Hz, 1H), 7.11 (d, *J*=8.7 Hz, 1H), 4.04-4.10 (m, 2H), 3.91 (s, 3H), 3.85 (s, 3H), 2.95-3.01 (m, 2H), 1.82-1.87 (m, 2H), 1.75 (br s, 2H), 1.67 (br s, 2H).
^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 167.5, 159.3, 157.5, 153.7, 151.2, 149.9, 149.1, 138.8, 129.3, 127.9, 125.2, 124.8, 124.3, 121.5, 120.9, 120.1, 111.9, 109.8, 55.8, 55.7, 45.1, 29.7, 28.0, 25.9, 25.2.

GSK-1.
Summary table of 1D and 2D NMR data.

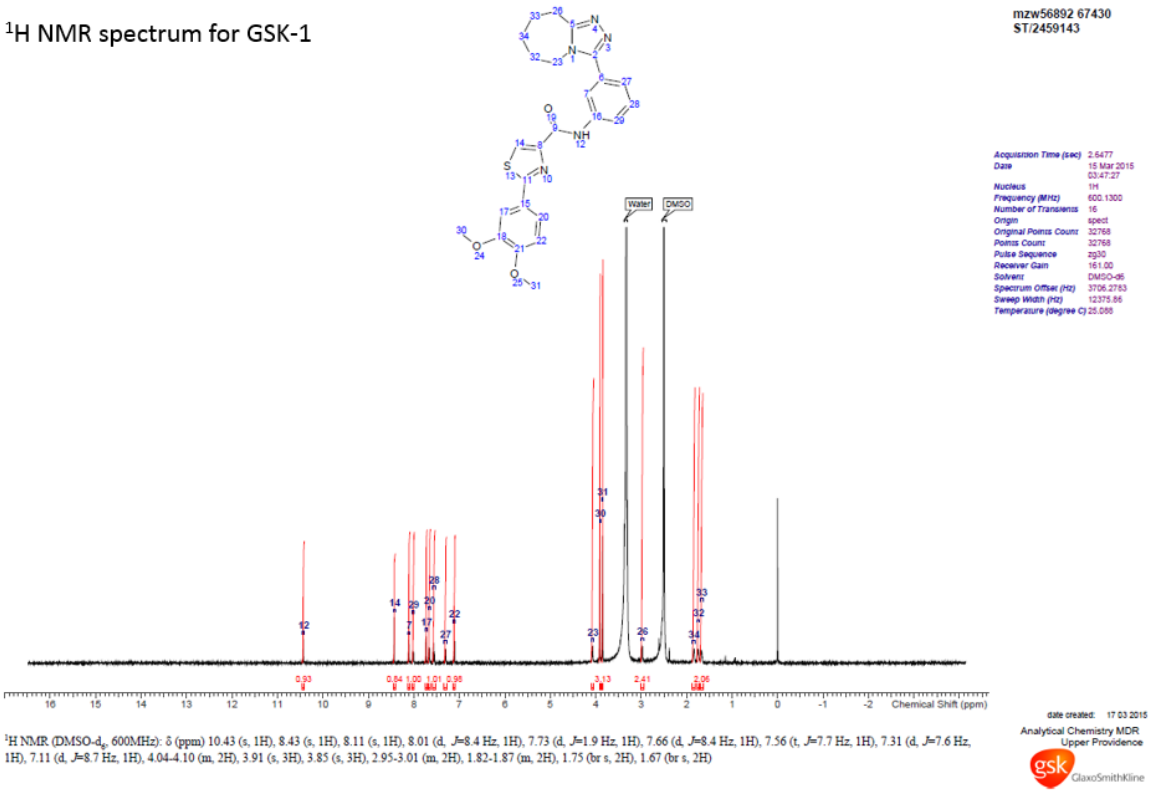


Atom#	C Shift	H Shift	H Multiplicity	H Volume	COSY	H HMBC	C HMBC
33	25.187	1.674	br s	-2.058	1.85, 2.98	2.98	
26	25.903	2.984	m	-2.414	1.67		29.74, 157.45, 25.19
32	28.021	1.746	br s	-2.113	1.85, 4.07	4.07	
34	29.741	1.845	m	-2.113	1.67, 1.75	2.98, 4.07	
23	45.131	4.072	m	-2.173	1.75		153.72, 157.45, 29.74, 28.02
31	55.697	3.846	s	3.131			111.86, 151.16
30	55.816	3.909	s	2.674			149.06, 109.77
17	109.766	7.732	d (1.89)	1.023	7.66	3.91, 7.11, 7.66	151.16, 167.48, 120.09, 125.20
22	111.860	7.112	d (8.69)	0.975	7.66	3.85	151.16, 125.20, 167.48, 109.77
20	120.093	7.661	d (8.41)	1.023	7.11, 7.73	7.73	151.16, 167.48, 109.77
7	120.889	8.116	s	1.002		8.01, 10.43	138.78, 153.72
29	121.542	8.014	d (8.36)	1.000	7.56	7.30, 10.43	120.89, 124.32
27	124.321	7.302	d (7.55)	0.964	7.56	8.01	121.54, 153.72
14	124.782	8.430	s	0.835			159.33, 167.48, 149.90
15	125.204					7.11, 7.73	
6	127.892					7.56	
28	129.273	7.565	t (7.74, 7.74)	1.014	7.30, 8.01		138.78, 127.89
16	138.779					7.56, 8.12, 10.43	
18	149.062					3.91	
8	149.902					8.43	
21	151.164					3.85, 7.11, 7.66, 7.73	
2	153.716					4.07, 7.30, 8.12	
5	157.450					2.98, 4.07	

	C Shift	H Shift	H Multiplicity	H Volume	COSY	H HMBC	C HMBC
9	159.329					8.43, 10.43	
11	167.478					7.11, 7.66, 7.73, 8.43	
12		10.432	s	0.930			121.54, 120.89, 159.33, 138.78

Supplemental Data – GSK-1 NMR spectra

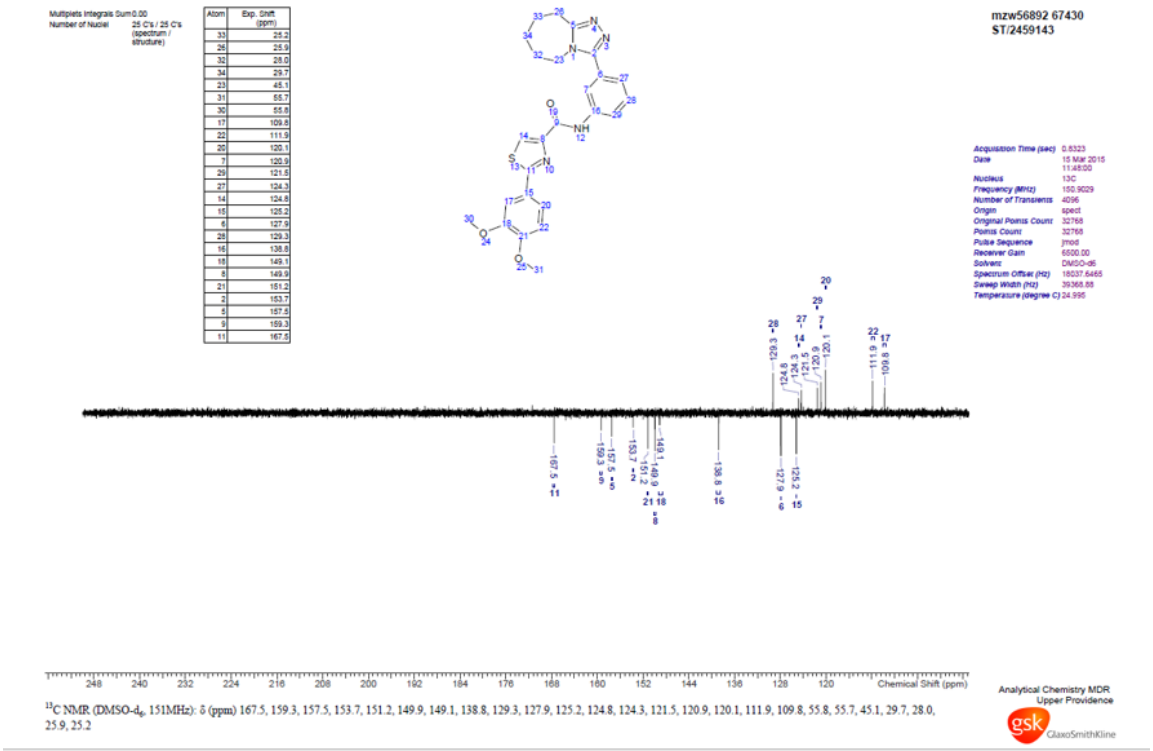
^{1}H NMR spectrum for GSK-1



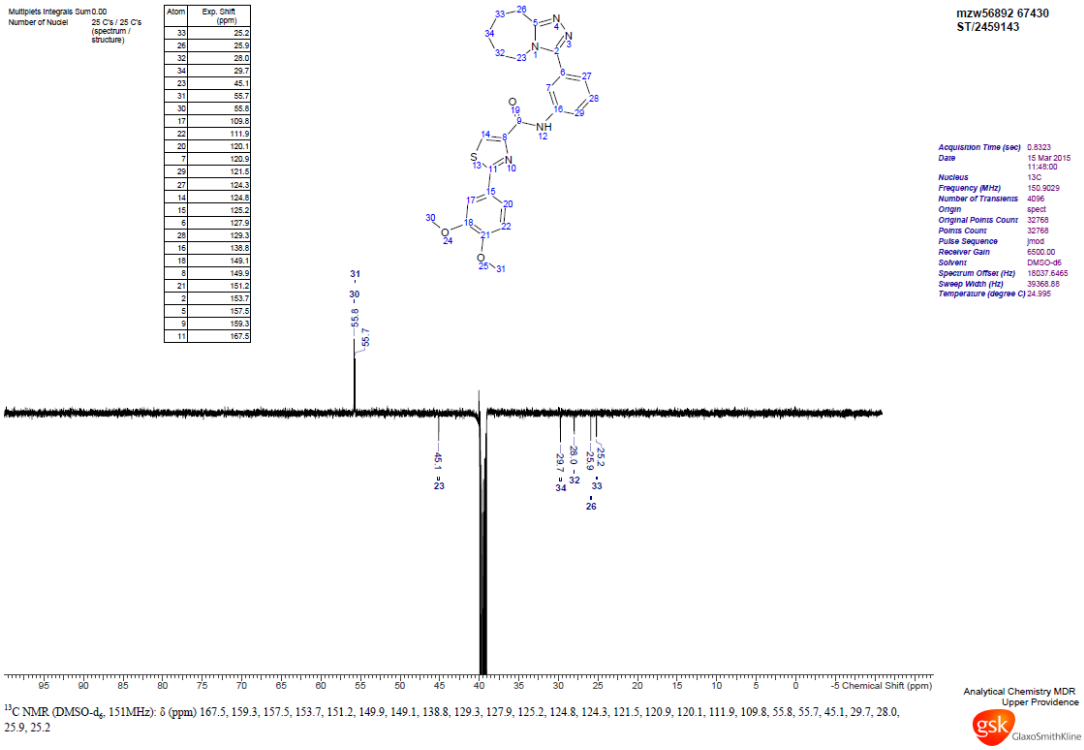
^{1}H NMR (DMSO-d_6, 600MHz): δ (ppm) 10.43 (s, 1H), 8.43 (s, 1H), 8.11 (s, 1H), 8.01 (d, *J*=8.4 Hz, 1H), 7.73 (d, *J*=1.9 Hz, 1H), 7.66 (d, *J*=8.4 Hz, 1H), 7.56 (t, *J*=7.7 Hz, 1H), 7.31 (d, *J*=7.6 Hz, 1H), 7.11 (d, *J*=8.7 Hz, 1H), 4.04-4.10 (m, 2H), 3.91 (s, 3H), 3.85 (s, 3H), 2.95-3.01 (m, 2H), 1.82-1.87 (m, 2H), 1.75 (br s, 2H), 1.67 (br s, 2H)

Supplemental Data – GSK-1 NMR spectra

^{13}C NMR spectrum for GSK-1

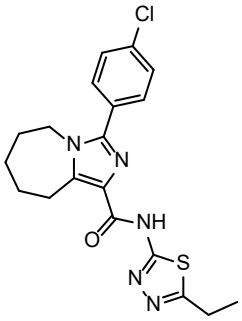


^{13}C NMR (DMSO-d_6, 151MHz): δ (ppm) 167.5, 159.3, 157.5, 153.7, 151.2, 149.9, 149.1, 138.8, 129.3, 127.9, 125.2, 124.8, 124.3, 121.5, 120.9, 120.1, 111.9, 109.8, 55.8, 55.7, 45.1, 29.7, 28.0, 25.9, 25.2



^{13}C NMR (DMSO-d_6, 151MHz): δ (ppm) 167.5, 159.3, 157.5, 153.7, 151.2, 149.9, 149.1, 138.8, 129.3, 127.9, 125.2, 124.8, 124.3, 121.5, 120.9, 120.1, 111.9, 109.8, 55.8, 55.7, 45.1, 29.7, 28.0, 25.9, 25.2

Supplemental Data – GSK-2 NMR spectra

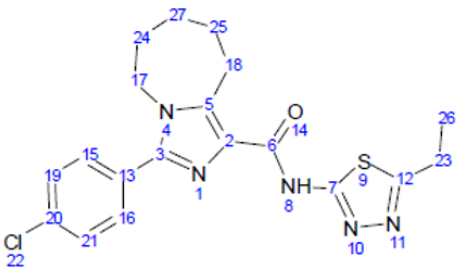


3-(4-Chlorophenyl)-N-(5-ethyl-1,3,4-thiadiazol-2-yl)-6,7,8,9-tetrahydro-5H-imidazo[1,5-a]azepine-1-carboxamide (ChemDiv cat # E711-0141).

^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 11.46 (br s, 1H), 7.64-7.68 (m, 2H), 7.60-7.64 (m, 2H), 4.05-4.18 (m, 2H), 3.01 (q, *J*=7.6 Hz, 2H), 1.83 (br s, 2H), 1.75 (br s, 2H), 1.67 (br s, 2H), 1.31 (t, *J*=7.6 Hz, 3H).

^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 165.2, 144.9, 134.1, 131.1, 128.8, 128.5, 46.8, 30.0, 27.8, 26.1, 23.8, 22.7, 13.9.

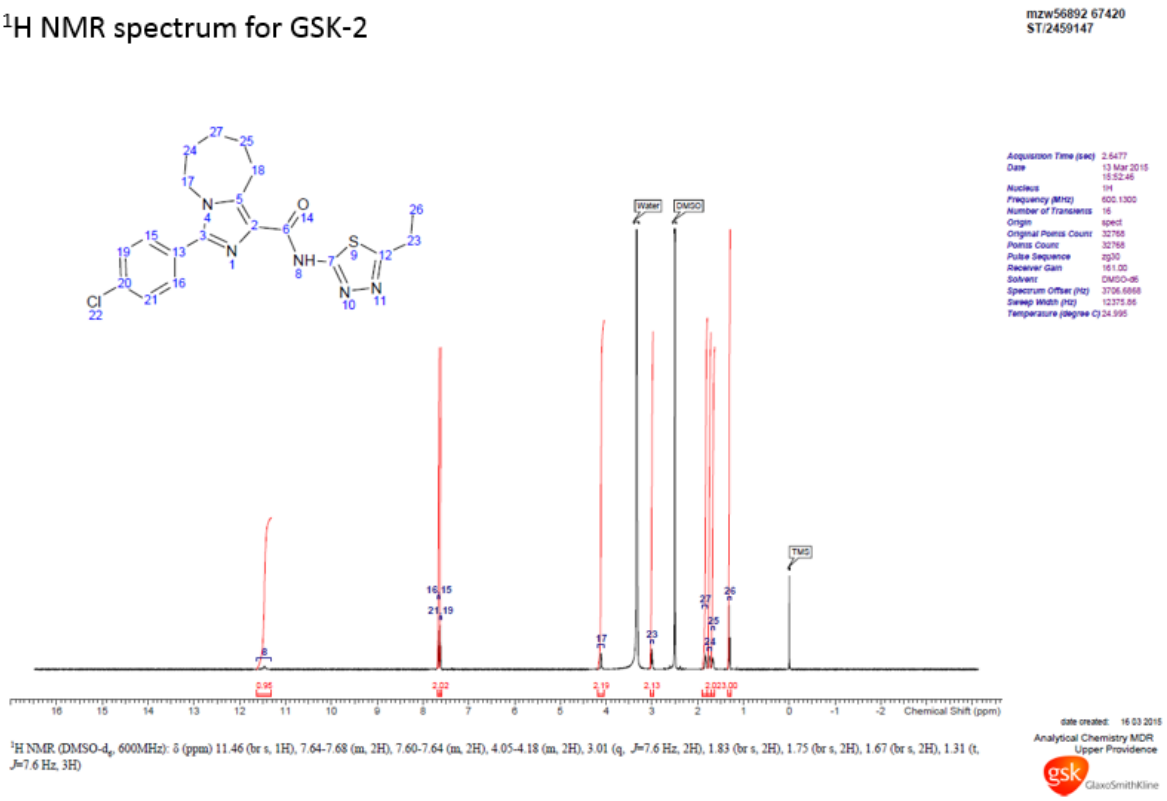
GSK-2.
Summary table of 1D and 2D NMR data.



Atom#	C Shift	H Shift	H Multiplicity	H Volume	COSY	H HMBC	C HMBC
26	13.865	1.312	t (7.55, 7.55)	3.000	3.01	3.01	165.21, 22.69
23	22.686	3.007	q (7.55, 7.55, 7.55)	-2.125	1.31	1.31	165.21, 13.86
18	23.777	3.339	u	-121.380	1.68		
25	26.062	1.676	br s	-2.022	1.84, 3.34		
24	27.782	1.753	br s	-2.117	1.84, 4.11	4.11	
27	29.964	1.838	br s	-2.208	1.68, 1.75	4.11	
17	46.787	4.113	m	-2.188	1.75		144.91, 27.78, 29.96
13	128.524					7.62	
19, 21	128.779	7.621	m	2.021			128.52
15, 16	131.088	7.660	m	2.025			144.91, 134.11
20, 2	134.106					7.66	
3	144.910					4.11, 7.66	
12	165.205					1.31, 3.01	
8		11.460	br s	0.865			

Supplemental Data – GSK-2 NMR spectra

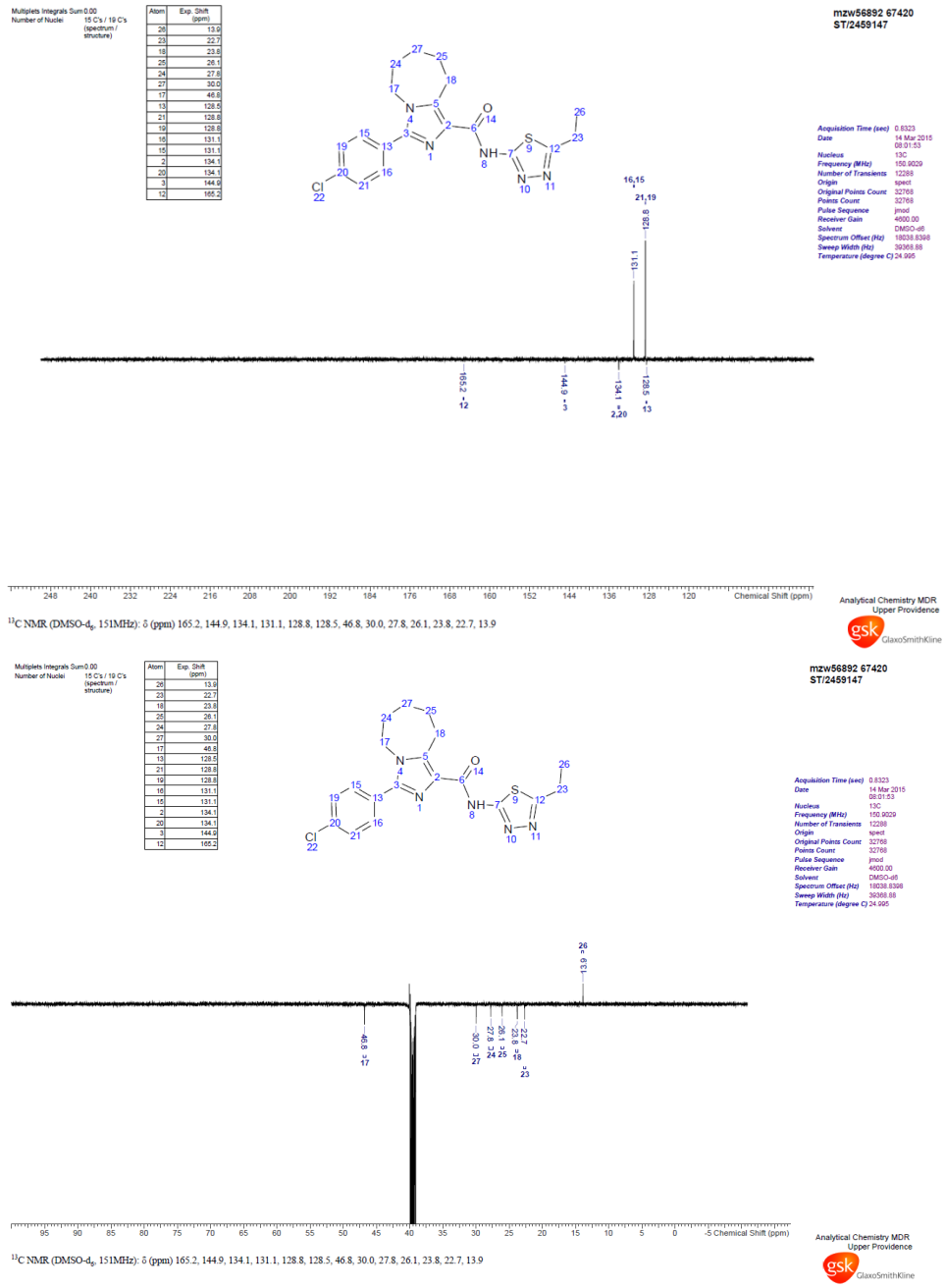
^{1}H NMR spectrum for GSK-2



^{1}H NMR (DMSO-d_6, 600MHz): δ (ppm) 11.46 (br s, 1H), 7.64-7.68 (m, 2H), 7.60-7.64 (m, 2H), 4.05-4.18 (m, 2H), 3.01 (q, *J*=7.6 Hz, 2H), 1.83 (br s, 2H), 1.75 (br s, 2H), 1.67 (br s, 2H), 1.31 (t, *J*=7.6 Hz, 3H)

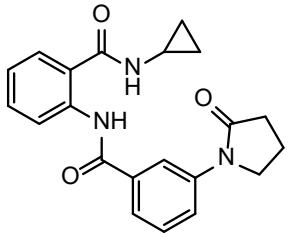
Supplemental Data – GSK-2 NMR spectra

^{13}C NMR spectrum for GSK-2



mzw56892 67420
ST/2459147
Chemical Shift (ppm)
Analytical Chemistry MDR
Upper Providence
GlaxoSmithKline
^{13}C NMR (DMSO-d_6, 151MHz): δ (ppm) 165.2, 144.9, 134.1, 131.1, 128.8, 128.5, 46.8, 30.0, 27.8, 26.1, 23.8, 22.7, 13.9

Supplemental Data – GSK-3 NMR spectra

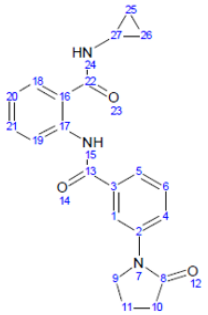


N-Cyclopropyl-2-{[3-(2-oxo-1-pyrrolidinyl)benzoyl]amino}benzamide
(Enamine cat # Z95754787)

^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 12.54 (s, 1H), 8.79-8.90 (m, 1H), 8.60 (d, *J*=8.3 Hz, 1H), 8.22 (s, 1H), 7.95 (br d, *J*=7.9 Hz, 1H), 7.79 (br d, *J*=7.9 Hz, 1H), 7.67 (br d, *J*=7.6 Hz, 1H), 7.61 (t, *J*=8.3 Hz, 1H), 7.56 (t, *J*=7.2 Hz, 1H), 7.19 (t, *J*=7.6 Hz, 1H), 3.92 (t, *J*=7.2 Hz, 2H), 2.90 (br dd, *J*=7.2, 3.4 Hz, 1H), 2.53-2.57 (m, 2H), 2.11 (quin, *J*=7.5 Hz, 2H), 0.71-0.76 (m, 2H), 0.61-0.65 (m, 2H).

^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 174.2, 170.0, 164.3, 140.2, 139.1, 135.2, 132.2, 129.3, 128.4, 122.8, 122.6, 121.6, 120.3, 120.3, 118.1, 48.0, 32.3, 23.2, 17.4, 5.7.

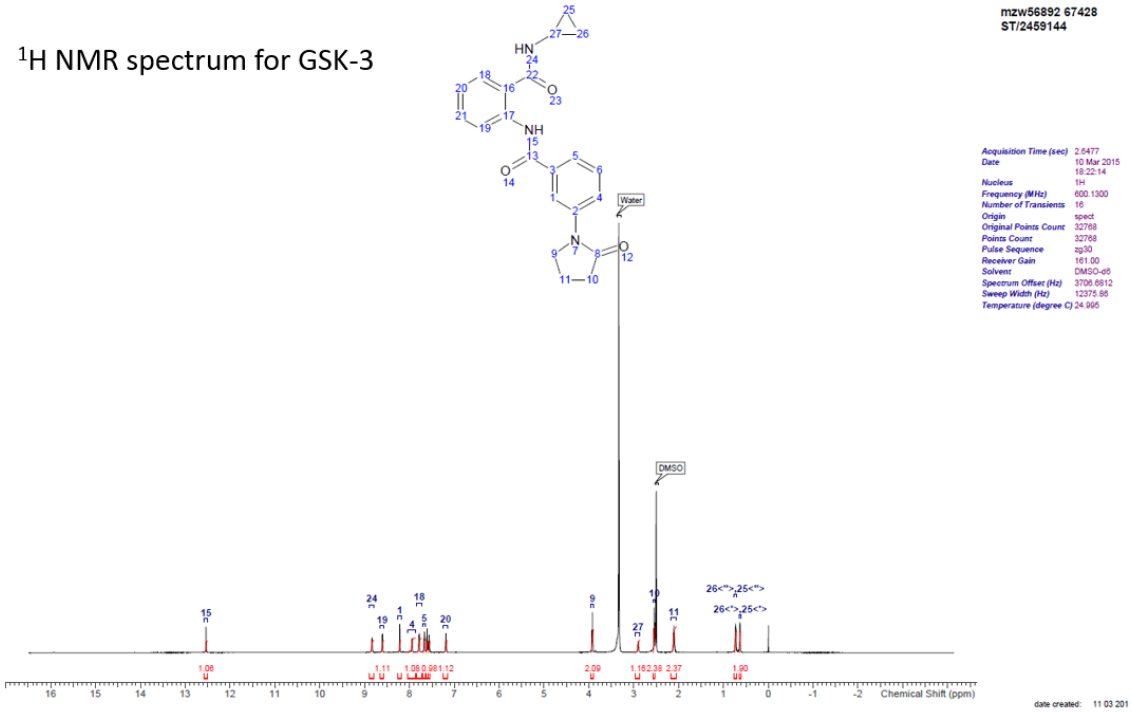
GSK-3.
Summary table of 1D and 2D NMR data.



Atom#	C Shift	H Shift	H Multiplicity	H Volume	COSY	H HMBC	C HMBC
25, 26	5.696					0.73	
25, 26	5.700	0.730	m	2.000	2.90		23.15, 5.70
25, 26	5.700	0.633	m	1.900	2.90	8.84	23.15
11	17.412	2.107	quin (7.46, 7.46, 7.46, 7.46	-2.368	2.55, 3.92	2.55, 3.92	32.33, 174.20, 48.02
27	23.152	2.900	br dd (7.18, 3.40)	1.157	0.63, 0.73, 8.84	0.63, 0.73, 8.84	
10	32.332	2.552	m	-2.519	2.11	2.11, 3.92	48.02, 17.41, 174.20
9	48.017	3.923	t (7.18, 7.18)	-2.095	2.11	2.11, 2.55	140.18, 174.20, 17.41, 32.33
1	118.122	8.218	s	1.082		7.67, 7.94	140.18, 164.28, 121.55
16	120.308					8.60	
19	120.320	8.604	d (8.31)	1.106	7.56	7.19, 7.56, 7.79, 12.54	169.96, 122.84, 120.31, 139.13
5	121.554	7.671	br d (7.55)	1.228	7.61	7.94, 8.22	164.28, 118.12, 122.57
4	122.573	7.944	br d (7.93)	1.083	7.61	7.61, 7.67	121.55, 118.12, 140.18
20	122.844	7.193	t (7.55, 7.55)	1.116	7.56, 7.79	8.60	120.32, 128.36, 139.13
18	128.361	7.787	br d (7.93)	1.069	7.19	7.19, 7.56	132.23, 139.13, 169.96, 120.32
6	129.325	7.607	m	1.204	7.67, 7.94		164.28, 135.17, 140.18, 122.57
21	132.231	7.564	m	0.974	7.19, 8.60	7.79, 12.54	139.13, 128.36, 120.32
3	135.173					7.61	
17	139.130					7.19, 7.56, 7.79, 8.60, 12.54	
2	140.181					3.92, 7.61, 7.94, 8.22	
13	164.282					7.61, 7.67, 8.22, 12.54	
22	169.958					7.79, 8.60, 8.84	
8	174.202					2.11, 2.55, 3.92	
24		8.839	m	1.121	2.90		169.96, 23.15, 5.70
15		12.537	s	1.056			164.28, 132.23, 139.13, 120.32

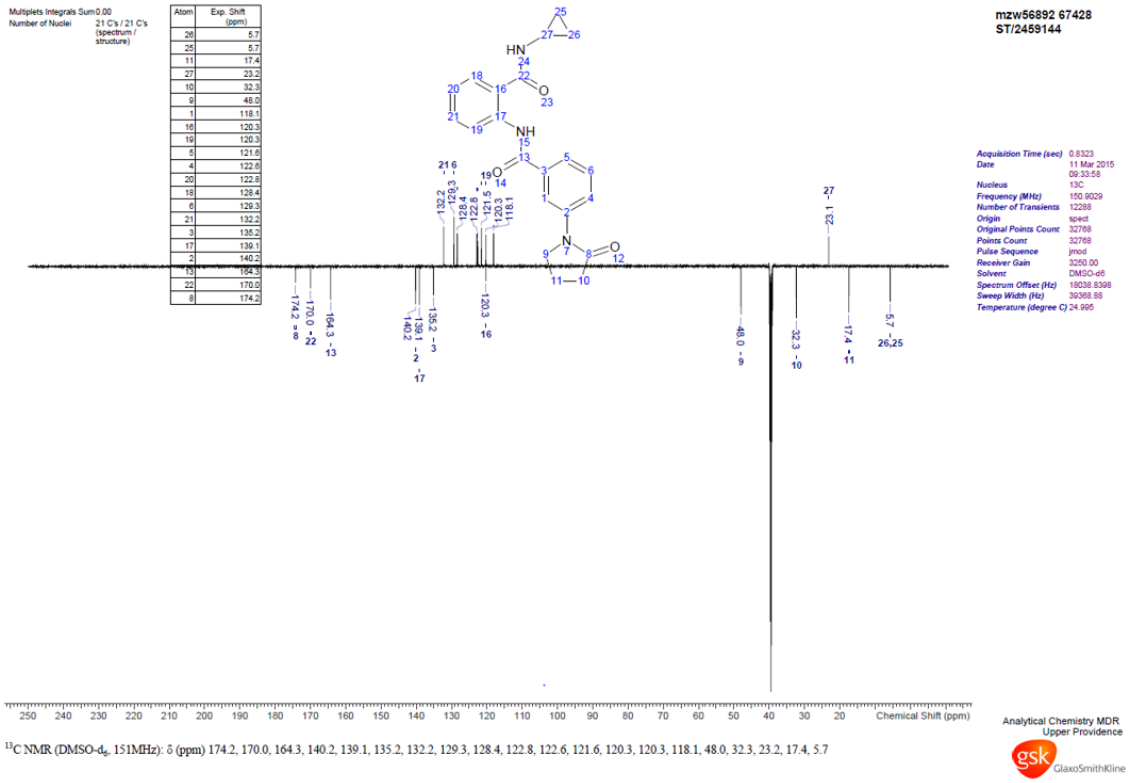
Supplemental Data – GSK-3 NMR spectra

^{1}H NMR spectrum for GSK-3



^{1}H NMR (DMSO-d_6, 600MHz): δ (ppm) 12.54 (s, 1H), 8.79-8.90 (m, 1H), 8.60 (d, *J*=8.3 Hz, 1H), 8.22 (s, 1H), 7.95 (br d, *J*=7.9 Hz, 1H), 7.79 (br d, *J*=7.9 Hz, 1H), 7.67 (br d, *J*=7.6 Hz, 1H), 7.61 (t, *J*=8.3 Hz, 1H), 7.56 (t, *J*=7.2 Hz, 1H), 7.19 (t, *J*=7.6 Hz, 1H), 3.92 (t, *J*=7.2 Hz, 2H), 2.90 (br dd, *J*=7.2, 3.4 Hz, 1H), 2.53-2.57 (m, 2H), 2.11 (quin, *J*=7.5 Hz, 2H), 0.71-0.76 (m, 2H), 0.61-0.65 (m, 2H)

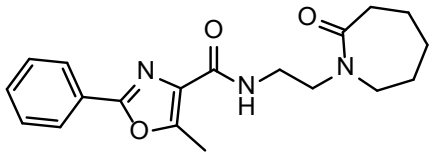
^{13}C NMR spectrum for GSK-3



Atom	Exp. Shift (ppm)
26	5.7
25	5.7
11	17.4
27	23.2
10	32.3
9	48.0
1	118.1
16	120.3
19	120.3
5	121.6
4	122.6
20	122.8
18	128.4
6	129.3
21	132.2
3	135.2
17	139.1
2	140.2
13	164.3
22	170.0
8	174.2

^{13}C NMR (DMSO-d_6, 151MHz): δ (ppm) 174.2, 170.0, 164.3, 140.2, 139.1, 135.2, 132.2, 129.3, 128.4, 122.8, 122.6, 121.6, 120.3, 120.3, 118.1, 48.0, 32.3, 23.2, 17.4, 5.7

Supplemental Data – GSK-4 NMR spectra

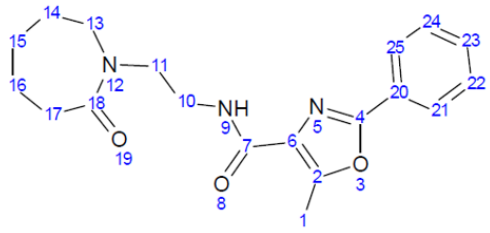


5-Methyl-N-[2-(2-oxo-1-azepanyl)ethyl]-2-phenyl-1,3-oxazole-4-carboxamide
(Enamine cat # Z407267740)

^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 8.19 (br t, *J*=5.1 Hz, 1H), 7.90-8.05 (m, 2H), 7.48-7.64 (m, 3H), 3.43-3.48 (m, 2H), 3.38-3.41 (m, 2H), 3.35-3.37 (m, 2H), 2.65 (s, 3H), 2.36-2.46 (m, 2H), 1.61-1.68 (m, 2H), 1.57 (br s, 2H), 1.48-1.55 (m, 2H).

^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 175.0, 161.3, 157.8, 152.4, 130.9, 130.2, 129.2, 126.3, 125.9, 49.2, 46.9, 37.2, 36.5, 29.2, 28.2, 23.0, 11.4.

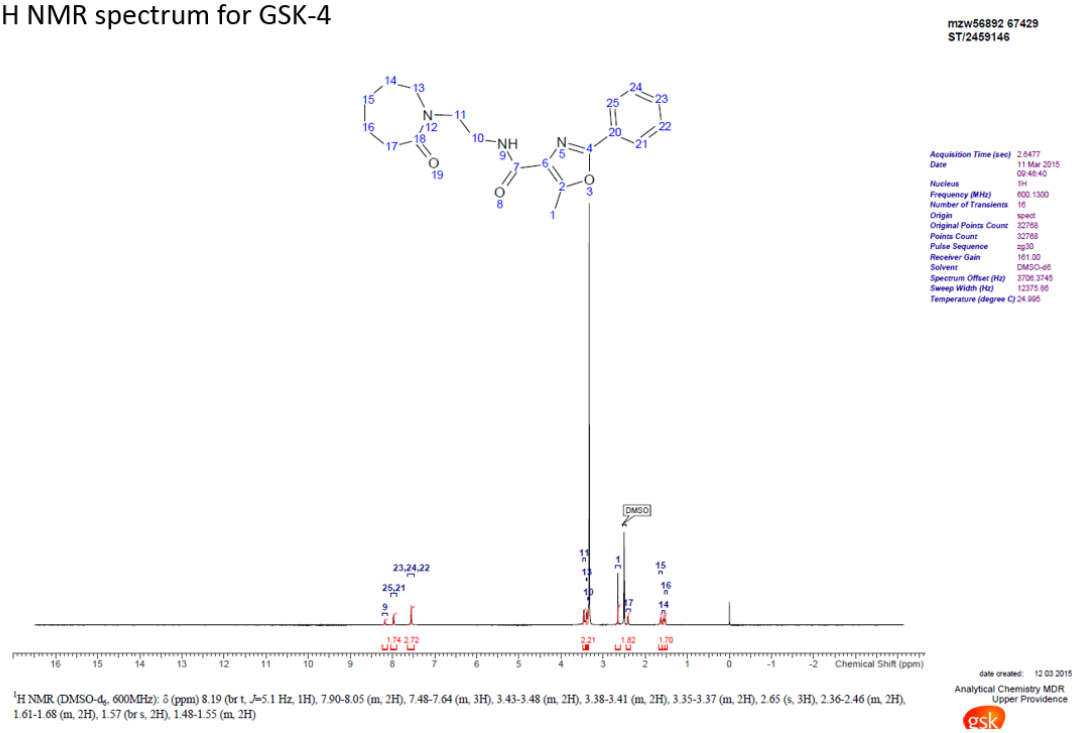
GSK-4.
Summary table of 1D and 2D NMR data.



Atom#	C Shift	H Shift	H Multiplicity	H Volume	COSY	H HMBC	C HMBC
1	11.444	2.649	s	3.000			130.20, 161.26, 152.43
16	22.965	1.536	m	-1.704	1.63, 2.41	1.57, 1.63, 2.41	36.54, 28.24, 174.97
14	28.244	1.566	br s	-1.653	3.40	1.54, 1.63	49.23, 22.97
15	29.243	1.630	m	-1.812	1.54	2.41, 3.40	49.23, 22.97, 36.54, 28.24
17	36.544	2.409	m	-1.824	1.54	1.54, 1.63	29.24, 22.97, 174.97
10	37.201	3.351	m	-2.213	3.46, 8.19	3.46, 8.19	161.26, 46.95
11	46.946	3.459	m	-2.446	3.35	3.35, 3.40	37.20, 49.23, 174.97
13	49.232	3.395	m	-2.308	1.57	1.57, 1.63, 3.46	174.97, 46.95, 29.24
21, 25	125.925	7.977	m	1.745	7.56		130.88, 157.75
20	126.311					7.56	
22, 24	129.213	7.559	m	1.767	7.98	7.55	126.31
6	130.204					2.65	
23	130.877	7.555	m	0.953		7.98	129.21
2	152.434					2.65	
4	157.753					7.98	
7	161.264					2.65, 3.35, 8.19	
18	174.966					1.54, 2.41, 3.40, 3.46	
9		8.186	br t (5.10, 5.10)	0.855	3.35		37.20, 161.26

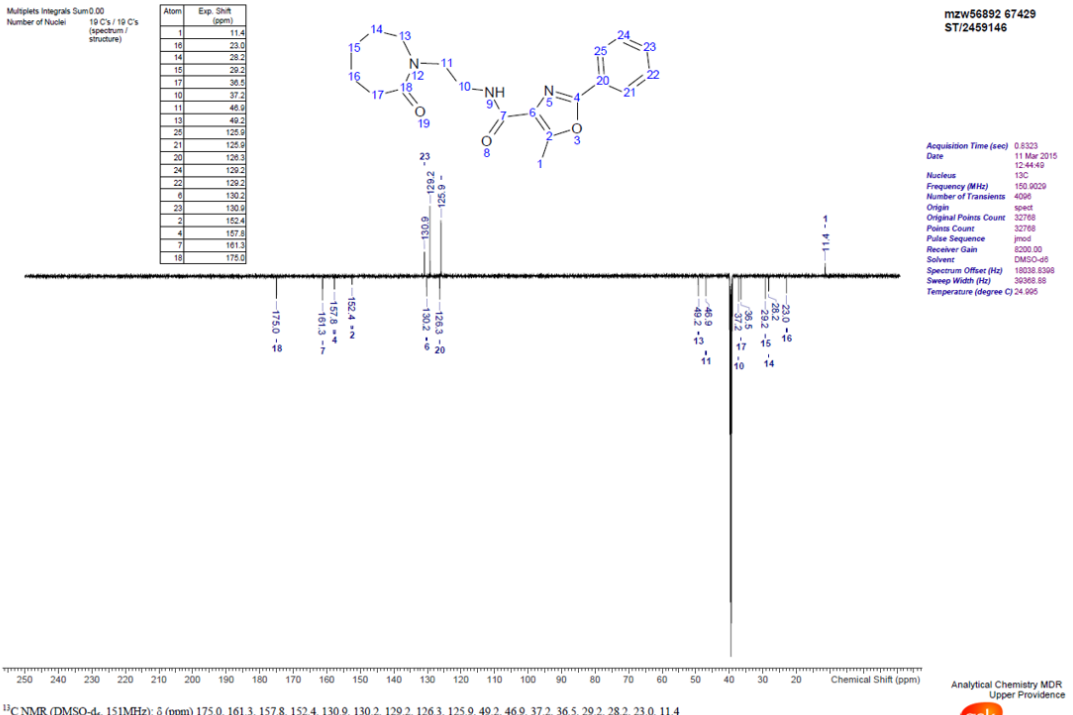
Supplemental Data – GSK-4 NMR spectra

^{1}H NMR spectrum for GSK-4



^{1}H NMR (DMSO-d_6, 600MHz): δ (ppm) 8.19 (br t, *J*=5.1 Hz, 1H), 7.90-8.05 (m, 2H), 7.48-7.64 (m, 3H), 3.43-3.48 (m, 2H), 3.38-3.41 (m, 2H), 3.35-3.37 (m, 2H), 2.65 (s, 3H), 2.36-2.46 (m, 2H), 1.61-1.68 (m, 2H), 1.57 (br s, 2H), 1.48-1.55 (m, 2H)

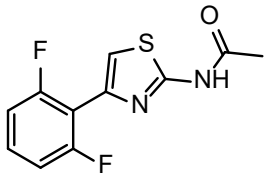
^{13}C NMR spectrum for GSK-4



Atom	Exp. Shift (ppm)
1	11.4
16	23.0
14	28.2
15	29.2
17	36.5
10	37.2
11	46.9
13	49.2
25	125.9
21	125.9
20	126.3
24	129.2
22	129.2
6	130.2
23	130.9
2	152.4
4	157.8
7	161.3
18	175.0

^{13}C NMR (DMSO-d_6, 151MHz): δ (ppm) 175.0, 161.3, 157.8, 152.4, 130.9, 130.2, 129.2, 126.3, 125.9, 49.2, 46.9, 37.2, 36.5, 29.2, 28.2, 23.0, 11.4

Supplemental Data – GSK-5 NMR spectra

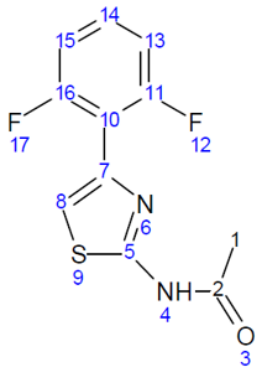


N-[4-(2,6-Difluorophenyl)-1,3-thiazol-2-yl]acetamide
(Enamine cat # Z384175608).

^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 12.30 (br s, 1H), 7.45-7.52 (m, 1H), 7.41 (s, 1H), 7.16-7.25 (m, 2H), 2.16 (s, 3H)

^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 168.7, 159.8, 157.6, 136.9, 130.4, 114.7, 112.7, 112.1, 22.5

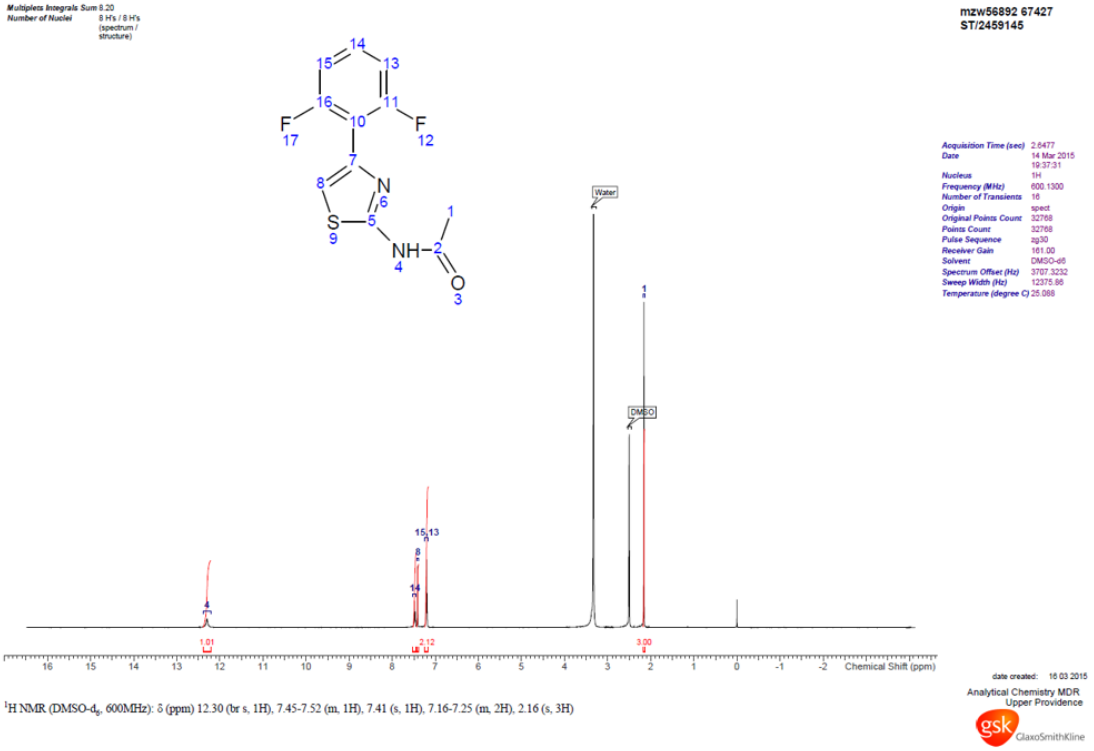
GSK-5.
Summary table of 1D and 2D NMR data.



Atom#	C Shift	H Shift	H Multiplicity	H Volume	COSY	H HMBC	C HMBC
1	22.475	2.162	s	3.000			157.61, 168.71
13, 15	112.065	7.206	m	1.517	7.48		159.77
10	112.749					7.41	
8	114.663	7.414	s	0.952			157.61, 136.92, 112.75
14	130.364	7.478	m	1.114	7.21		159.77
7	136.916					7.41	
5	157.609					2.16, 7.41	
11, 16	159.773					7.21, 7.48	
2	168.708					2.16	
4		12.300	br s	1.010			

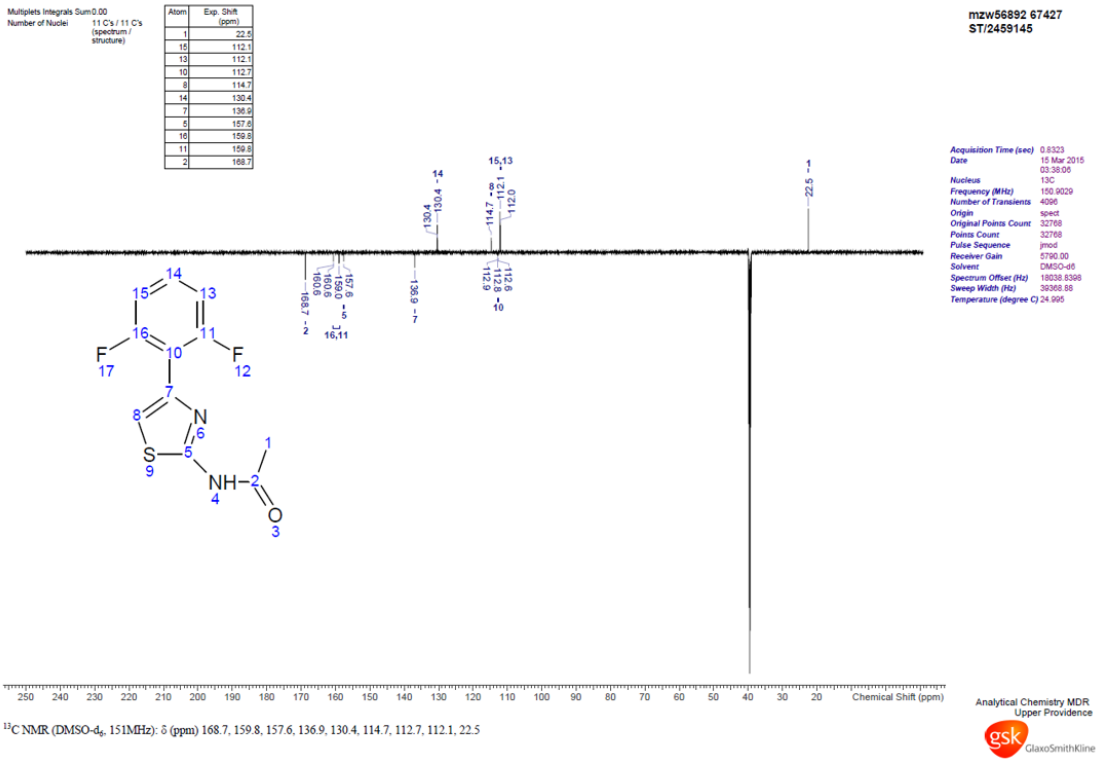
Supplemental Data – GSK-5 NMR spectra

^{1}H NMR spectrum for GSK-5



^{1}H NMR (DMSO-d_6, 600MHz): δ (ppm) 12.30 (br s, 1H), 7.45-7.52 (m, 1H), 7.41 (s, 1H), 7.16-7.25 (m, 2H), 2.16 (s, 3H)

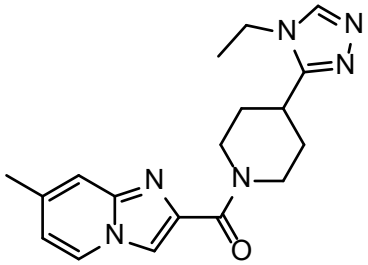
^{13}C NMR spectrum for GSK-5



Atom	Exp. Shift (ppm)
1	22.5
15	112.1
13	112.1
10	112.7
8	114.7
14	130.4
7	136.9
5	157.6
16	159.8
11	159.8
2	168.7

^{13}C NMR (DMSO-d_6, 151MHz): δ (ppm) 168.7, 159.8, 157.6, 136.9, 130.4, 114.7, 112.7, 112.1, 22.5

Supplemental Data – GSK-6 NMR spectra



[4-(4-Ethyl-4H-1,2,4-triazol-3-yl)-1-piperidinyl](7-methylimidazo[1,2-a]pyridin-2-yl)methanone (Enamine Z1082942462).

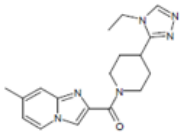
^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) 8.45 (d, *J*=7.0 Hz, 1H), 8.43 (s, 1H), 8.23 (s, 1H), 7.38 (s, 1H), 6.82 (dd, *J*=1.1, 7.0 Hz, 1H), 5.14 (br s, 1H), 4.55 (br s, 1H), 4.02 (q, *J*=7.2 Hz, 2H), 3.37 (br s, 1H), 3.20 - 3.14 (m, 1H), 3.00 (br s, 1H), 2.36 (s, 3H), 1.93 (br s, 1H), 1.93 (br s, 1H), 1.74 (br s, 1H), 1.74 (br s, 1H), 1.35 (t, *J*=7.3 Hz, 3H)

^{13}C NMR (DMSO-d_6, 151 MHz): δ (ppm) 162.48, 155.55, 143.62, 143.10, 140.09, 136.34, 126.33, 115.94, 115.55, 115.44, 45.73, 41.75, 38.24, 31.25, 31.02, 30.46, 20.78, 15.99

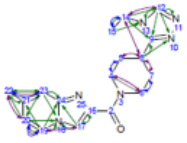
GSK-6.

Summary table of 1D and 2D NMR data.

Atom (#)	13C shift (ppm)	X Shift (ppm)	1H shift (ppm)	Atom (label)	ROESY or NOESY (to or from H)	HMBC (from H)	HMBC (to C)	XHMBC (to X)
2	162.5							
4	45.7		5.14	"	6, 4, 6			
4	45.7		3.36	'	6, 6, 4			
5	31.3		1.75	'				
5	31.3		1.92	"	8			
6	41.8		4.55	"	6, 4, 4			
6	41.8		3.01	'	4, 6, 4			
7	30.5		1.74	'	7			
7	30.5		1.94	"	7, 8			
8	31.0		3.17		5, 7, 14		9	10
9	155.6					8, 14, 12		
10		313.7				8, 12		
11		315.7				12		
12	143.1		8.44		14	14	9	13, 10, 11
13		174.8				15, 14, 12		
14	38.2		4.02		8, 12	15	15, 12, 9	13
15	16.0		1.35			14	14	13
16	140.1					17		
17	115.9		8.23		19		16, 24	18
18		198.8				20, 23, 17, 19		
19	126.3		8.45		20, 17	20	20, 21, 24	18
20	115.6		6.82		22, 19	23, 19, 23	22, 23, 19	18
21	136.3					22, 19		
22	20.8		2.36		20, 23	20, 23	23, 21	
23	115.4		7.38		22	22, 20, 20	22, 20, 24	18
24	143.6					23, 17, 19		



Structure Diagram



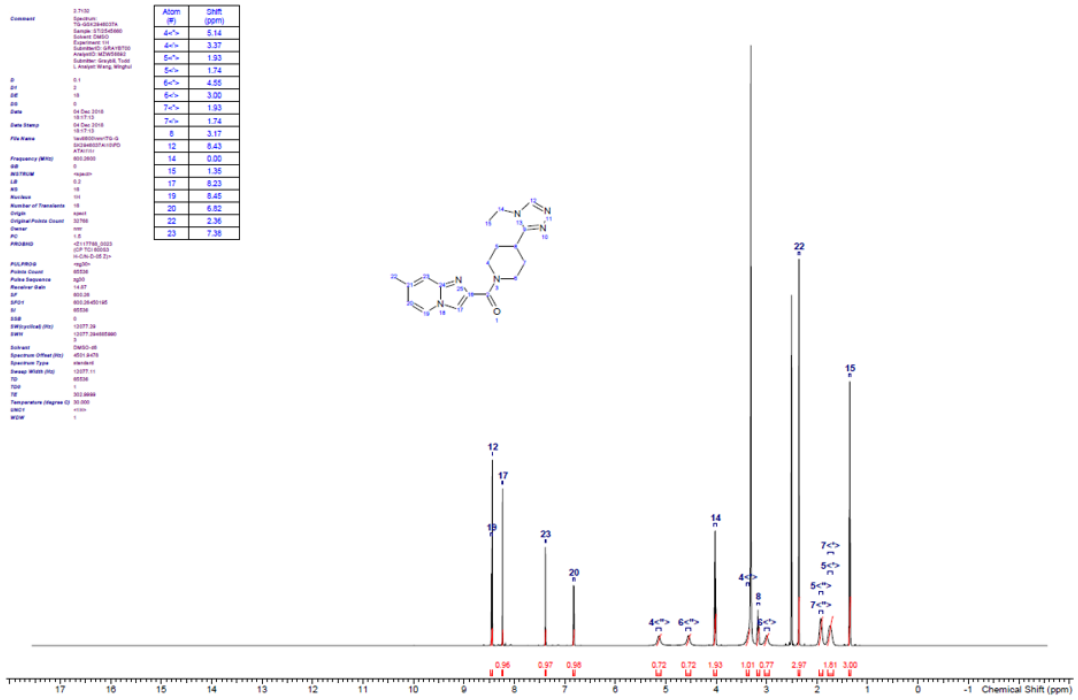
Key: Correlation Arrows (if present): green: HMBC, blue: COSY, purple: ROESY/NOESY, red: check in spectra.

Atom # / NMR Correlations

Supplemental Data – GSK-6 NMR spectra

^{1}H NMR spectrum for GSK-6

Atom (#)	Shift (ppm)
4<'>	5.14
4<'>	3.37
5<'>	1.93
5<'>	1.74
6<'>	4.55
6<'>	3.00
7<'>	1.93
7<'>	1.74
8	3.17
12	8.43
14	0.00
15	1.36
17	8.23
19	8.45
20	6.82
22	2.36
23	7.38



^{13}C NMR spectrum for GSK-6

Atom (#)	Shift (ppm)
2	162.5
4	45.7
5	31.3
6	41.8
7	30.5
8	31.0
9	155.6
12	143.1
14	38.2
15	16.0
16	140.1
17	115.9
19	126.3
20	115.6
21	136.3
22	20.8
23	115.4
24	143.6