Supplementary information

Materials and Methods

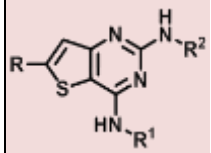
DNA microarray analysis

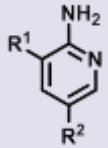
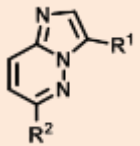
Late stage gametocytes were enriched by density centrifugation using Nycoprep 1.077 cushions (Axis-Shield) prior to RNA isolation as described.[1] DNA microarray was performed using 3-12 µg total RNA for reverse transcription and aminoallyl incorporation for each sample to be used in a reference design (reference pool of equal amounts of all cDNA samples coupled to Cy3 hybridized to samples coupled to Cy5, 17 h at 65ºC while rotating) performed on *P. falciparum* custom Agilent 60-mer 8x15k arrays (AMADID#037237,[2]). Post washing, arrays were scanned on a GenePix 4000B scanner (10 µm resolution) at wavelengths of 532 nm (Cy3) and 635 nm (Cy5). Signal intensities that passed Genepix standard background filters (P<0.05) were normalized by robustspline and GQuantile using limma Log_2 (Cy5/Cy3) expression values were used to calculate the log fold change in gene expression (log_2(T/UT)) with differential expression set at >0.5 or <-0.5. Data were clustered hierarchically according to Euclidean distance with average linkage clustering using TIGR MeV 4.9.0. Pearson correlation coefficients were calculated and visualized using the corrplot package in R (v3.2.3). Gene ontology annotations were obtained from the Gene Ontology Consortium (http://www.geneontology.org/) and combined with genes involved in different metabolic pathways from the Malaria Parasite Metabolic Pathways (MPMP) database (http://mpmp.huji.ac.il/) and INTERPRO domains for *P. falciparum* proteins were obtained from UniProt (http://www.uniprot.org/). Gene set enrichment analysis (GSEA) was applied to determine the enriched processes using GSEA v2.2.4

(http://www.broad.mit.edu/gsea/index.jsp) (P<0.05, FDR<0.1%) and interaction networks visualized with Cytoscape v3.5.0.

Supplementary results

Table S1: Biological profiles of the evaluated compounds. The DTPs series are indicated in pink, the APs in purple and the IMPs in orange. Colour intensity represents decreasing *in vitro* potency against *P. falciparum* asexual stages, early (stage II/III) gametocytes and late (stage IV/V) gametocytes. Gametocyte IC_{50}'s represents the lowest of each value obtained for the luciferase reporter or ATP bioluminescence assays (italised values). Data are representative of at least two biological experiments, each performed in technical triplicates, ± SEM.

Series scaffold	Compound	Pf asexual IC_{50} (nM)		RI	Ref	IC_{50} CHO cells (µM)	SI (CHO:NF54)	Gametocyte IC_{50} (nM)							SI (CHO:LG)	Ref
								Stg II/III			Stg IV/V			Stg V		
		NF54	K1					Mean		SEM	Mean		SEM			
 **diaminothienyl-pyrimidines**	MMV668434	42.54	ND	ND	3	16.87	396.53	513.70		ND	105.20		ND	ND	160.36	
	MMV666632	28.20	25.85	0.92	4	4.12	146.08	404.65	±	140.25	642.70	±	15.66	ND	6.41	
	MMV670771	35.60	ND	ND		1.81	ND	4182.50	±	94.50	1091.00	±	71.00	ND	1.66	
	MMV668436	163.91	ND	ND	3	2.10	12.81	781.60	±	82.90	1519.00	±	104.00	ND	1.38	
	MMV670997	20.08	33.04	1.65		ND	ND	442.30	±	50.30	2193.00	±	716.00	ND	ND	
	MMV667613	115.95	ND	ND	3	>260	>2200	ND		ND	*2390.00*		ND	ND	>100	
	MMV667482	26.09	86.96	3.33		4.32	165.60	2960.00	±	15.00	2935.00	±	394.00	ND	1.47	
	MMV668308	194.24	ND	ND	4	19.66	101.22	6525.00	±	742.00	*5840.00*		ND	ND	3.37	
	MMV669340	46.76	137.81	2.95	3	86.31	1845.91	ND		ND	ND		ND	ND	ND	
	MMV672639	167.60	ND	ND		>260	>2200	ND		ND	ND		ND	ND	ND	
	MMV672720	766.70	ND	ND		220.94	288.17	ND		ND	ND		ND	ND	ND	
	MMV672965	2380.00	ND	ND		>260	>2200	ND		ND	ND		ND	ND	ND	
	MMV642943	5.73	5.15	0.96	5	18.90	3512.36	134.00		ND	66.00	±	6.40	125.60	286.36	6
	MMV674192	9.17	7.00	0.76	6	196.00	21373.64	415.90	±	42.10	45.24	±	9.09	ND	4332.45	6
	MMV642944	20.16	8.37	0.83	7	282.00	27803.44	46.80	±	22.80	51.90	±	8.50	96.80	5433.53	
	MMV643110	23.24	16.42	0.82	6	70.91	3538.71	122.10	±	12.70	71.51	±	2.38	92.70	991.61	
	MMV642942	10.17	8.64	0.85	7	254.00	24980.07	550.00		ND	134.70	±	1.20	ND	1885.67	
	MMV668647	21.47	19.61	0.91	6	40.94	1906.44	322.90		ND	136.90	±	0.50	ND	299.05	6
	MMV390048	22.12	17.79	0.80	8	254.00	11484.97	214.60	±	16.70	140.30	±	11.40	897.00	1810.41	9

 **2-amino-pyridines**	MMV673927	15.00	ND	ND		147.12	9808.00	697.30	±	102.00	146.05	±	26.85	ND	1007.33	
	MMV666810	5.42	4.97	0.92	5	226.00	41663.19	602.50	±	87.90	178.70	±	7.80	ND	1264.69	6
	MMV670930	14.27	14.01	0.98	7	241.00	16886.78	639.80	±	435.00	189.90	±	28.10	ND	1269.09	
	MMV394902	18.60	19.88	1.07	8	27.60	1483.59	919.55	±	185.45	208.60	±	4.90	ND	132.31	
	MMV642941	53.02	50.23	0.95		249.00	4695.92	619.85	±	18.75	224.50		ND	ND	1109.13	
	MMV642990	13.95	13.12	0.94	6	ND	ND	754.05	±	13.55	236.95	±	41.75	ND	ND	6
	MMV670401	42.11	32.75	0.78		157.63	3743.06	1168.50	±	80.50	238.40	±	5.80	ND	661.20	
	MMV668808	93.76	73.25	0.78	7	>260	>2200	1390.00	±	136.00	342.20	±	18.50	ND	>700	
	MMV668809	38.08	27.24	0.72	7	246.22	6465.98	2433.00	±	48.00	432.40	±	27.00	ND	569.43	
	MMV670402	25.74	19.65	0.76		48.43	1881.84	1319.50	±	28.50	440.90	±	82.00	ND	109.84	
	MMV672643	42.40	39.75	0.94		190.40	4490.92	1071.00		ND	460.00	±	97.00	ND	413.91	
	MMV668648	6.02	ND	ND	6	180.52	30000.90	731.35	±	413.65	536.20	±	283.60	ND	336.67	
	MMV675081	24.42	26.09	1.07	6	178.00	7287.68	168.00	±	14.50	845.00	±	107.00	ND	210.65	6
	MMV668807	26.16	19.54	0.75	7	>260	>2200	1835.00	±	278.00	901.25	±	108.75	ND	>250	
	MMV674796	239.00	ND	ND		>260	>2200	ND		ND	1574.00	±	21.00	ND	>150	
	MMV674594	89.06	91.84	1.03	6	278.00	3121.48	649.00		ND	1860.00	±	770.00	ND	149.46	6
	MMV674333	189.12	206.92	1.09		222.00	1173.86	ND		ND	1986.00	±	250.00	ND	111.78	
	MMV670393	248.42	205.22	0.83		242.35	975.56	3790.00		ND	4267.00		ND	ND	56.80	
	MMV390535	637.78	500.90	0.79	8	>260	>2200	ND		ND	22800.00	±	8000.00	ND	>10	
	MMV034136	574.69	500.94	0.87	8	>260	>2200	1793.00	±	556.00	ND		ND	ND	ND	
	MMV390394	980.13	942.34	0.96	5	>260	>2200	ND		ND	ND		ND	ND	ND	
	MMV674578	2180.00	ND	ND		>260	>2200	ND		ND	ND		ND	ND	ND	
	MMV674579	2180.00	ND	ND		>260	>2200	ND		ND	ND		ND	ND	ND	
	MMV674944	2440.00	ND	ND		>260	>2200	ND		ND	ND		ND	ND	ND	
 **imidazo-pyridazines**	MMV669810	0.45	0.37	0.89		221.00	527537.46	2.00	±	0.80	1.40	±	0.05	1.20	157857.14	
	MMV669286	0.88	0.53	0.60	10	2.42	2740.51	4.10	±	2.40	3.00	±	0.13	34.20	805.67	
	MMV672652	0.56	ND	ND	10	2.90	3222.22	10.80	±	0.90	3.30	±	0.19	18.10	878.79	
	MMV652103	7.25	6.32	0.87	11	234.00	32269.43	59.60	±	12.98	27.00	±	2.40	133.30	8666.67	
	MMV674850	2.65	2.43	0.92	12	221.00	83344.81	4.47	±	3.63	28.70	±	0.24	208.80	7700.35	12
	MMV674766	7.86	ND	ND	12	134.00	16750.00	93.90	±	16.70	66.40	±	4.60	262.90	2018.07	12
	MMV675615	4.47	3.67	0.80		17.90	3907.09	163.20	±	36.20	72.10	±	4.60	104.20	248.27	12
	MMV666620	11.59	9.53	0.92	11	19.34	1859.64	68.40	±	23.80	78.90	±	3.20	428.00	245.12	

	MMV672925	10.20	0.94	0.09	10	212.00	20783.51	693.35		ND	476.80		ND	ND	444.63
	MMV670815	417.40	ND	ND	12	>260	>2200	ND		ND	*654.75*	±	46.25	ND	>350
	MMV672653	35.80	ND	ND		254.00	7094.97	ND		ND	*907.27*	±	237.90	ND	279.96
	MMV674132	43.71	45.89	1.05	12	219.00	5010.40	2604.00		ND	1453.50	±	70.50	ND	150.67
	MMV665078	59.89	45.52	0.76	11	240.00	4007.13	ND		ND	1462.00	±	193.00	ND	164.16
	MMV669289	95.69	81.68	0.85	10	>260	>2200	ND		ND	1489.00	±	10.00	ND	>150
	MMV675704	80.72	ND	ND		202.00	2502.63	ND		ND	1789.00	±	371.50	ND	112.91
	MMV670654	35.40	30.34	0.86	10	54.10	1528.34	35696.00	±	3711.00	7993.00		ND	ND	6.77
	MMV670656	42.84	24.80	0.58		27.60	644.23	ND		ND	ND		ND	ND	ND
	MMV652459	282.58	246.21	0.87		>260	>2200	ND		ND	ND		ND	ND	ND
	MMV674326	372.04	ND	ND		>260	>2200	ND		ND	ND		ND	ND	ND
	MMV652454	496.28	415.19	0.84	11	>260	>2200	ND		ND	ND		ND	ND	ND
	MMV670225	876.55	ND	ND	12	>260	>2200	ND		ND	ND		ND	ND	ND
	MMV639846	963.97	873.14	0.91	11	209.35	217.17	ND		ND	ND		ND	ND	ND
	MMV666812	2339.18	2339.18	1.00	12	>260	>2200	ND		ND	ND		ND	ND	ND

[a]Previously published asexual stage data included as per references, for comparative purposes.

[b]RI = resistance index = ratio of the IC_{50} values of resistant to susceptible strain.

[c]SI = selectivity index = ratio of IC_{50} against asexual *P. falciparum* to IC_{50} against mammalian cells.

Table S2: Origin and drug resistance genotypes of clinical isolates from southern African producing gametocytes

Strain	Origin	Resistance phenotype	Resistance mechanism (genotype)	Gametocyte production (Y; %)
KF_01	Mozambique	Pyrimethamine	*pfdhfr, pfdhps, pfcrt*	2.4 ± 0.3% (n=4)
TD_01	Mozambique	Pyrimethamine	*Pfdhfr, pfcrt*	0.9 ± 0.1% (n=4)
SB_04	Malawi	Pyrimethamine, mefloquine	*pfdhfr, pfdhps, pfmdr1* (mixed)	1.4% (n=1)
SB_05	Mozambique	Pyrimethamine	*pfdhfr, pfdhps, pfcrt*	1.1 ± 0.4% (n=4)
SB_07	Malawi	Pyrimethamine, methylene blue	*pfdhfr, pfdhps, pfcrt*	0.5% (n=1)
JZA15	NA	Pyrimethamine	*Pfdhfr, pfmdr1*	1.1% (n=1)
JZA20	NA	Pyrimethamine	*pfdhfr, pfdhps*	0.8% (n=1)
JZA25	NA	Pyrimethamine	*pfcrt, pfdhfr, pfdhps*	0.8% (n=1)
JZA30	NA	Pyrimethamine, Mefloquine, Atovaquone	*pfdhfr, pfdhps, pfcrt*	0.8% (n=1)
JZA39	NA	Pyrimethamine, Lumefantrine	*pfdhfr*	1.3% (n=1)

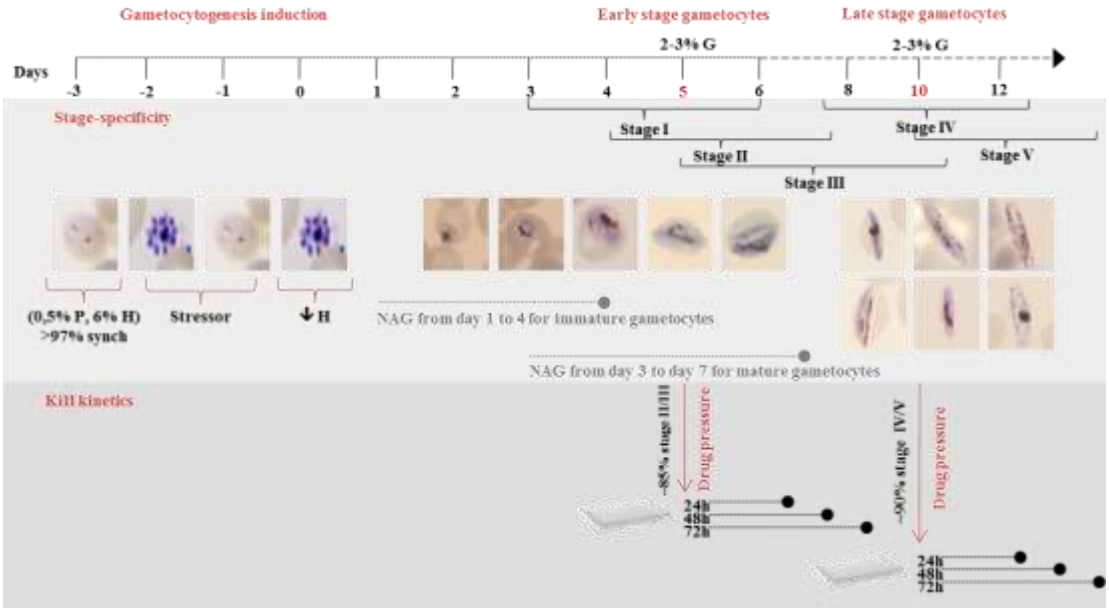


Figure S1. Stage-specific gametocyte production and speed-of-action assay. Asexual parasites (0.5% parasitemia, 6% hematocrit) were sorbitol synchronized for two consecutive cycles to ensure a highly synchronized (>97%) ring population on day -3. Initiation of gametocytogenesis was induced on day 0 by a decrease in the haematocrit. Asexual elimination was achieved with NAG treatment from day 1 to 4 for early gametocytes and day 3 to 8 for mature gametocyte. Assays were performed on day 5 and 10 for early and late gametocytes, and incubated for 24, 48, and 72 h. P: parasitemia, H: hematocrit, G: gametocytemia.

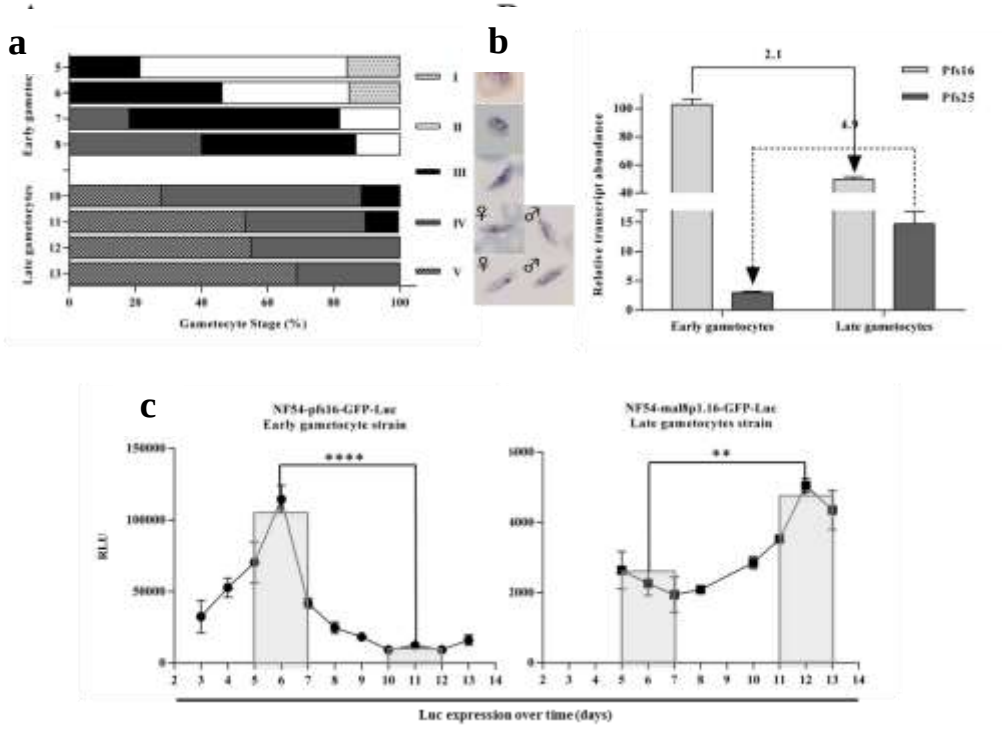


Figure S2. Stage-specific metrics. **(a)** Gametocyte stage confirmation and percentage using Giemsa smears over the assay time periods. For the day 5 and 10 populations, data are from >49 individual experiments; the subsequent daily evaluation was performed once. **(b)** Semi-quantitative real time PCR confirmation of the expression of the early gametocyte marker (*pfs16*) and late gametocyte marker (*pfs25*) on the day of assay (day 5 for early gametocytes and day 10 for late gametocytes ($P<0.05$). **(c)** Assessment of the luciferase expression (RLU) throughout gametocytogenesis of the two transgenic lines used (NF54-*pfs16*-GFP-Luc: $P<0.0001$ and NF54-*mal8p1.16*-GFP-Luc: $P<0.001$, n=3, ± SEM).

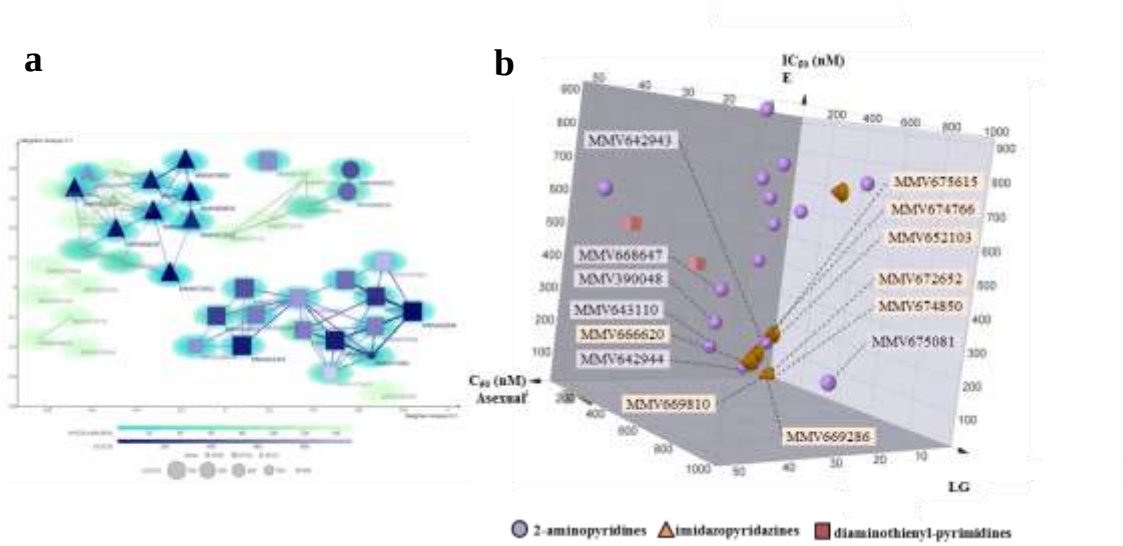


Figure S3: Pan-reactivity of active compounds against all stages of *P. falciparum* parasites tested. **(a)** SALI plot of pan-reactivity associated with chemical features for each of the 66 compounds screened. Pairwise late (IV/V) stage gametocyte activity to structural feature (SkelSphere) analysis was performed with activity cliff analysis (Osiris DataWarrior V 4.2.2) on all three series at a stringency of 80% in structural characteristics. **(b)** Comparison of the pan-reactive nature of hit compounds targeting asexual parasites (z-axis, <100 nM IC_{50}), early (>95% II/III; y-axis, <1 µM IC_{50}) and late stage (>95% IV/V; x-axis, <1 µM IC_{50}) gametocytes. Data are representative of at least three biological experiments, each performed in technical triplicates.

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