

Supplementary Material

1 Supplementary figures

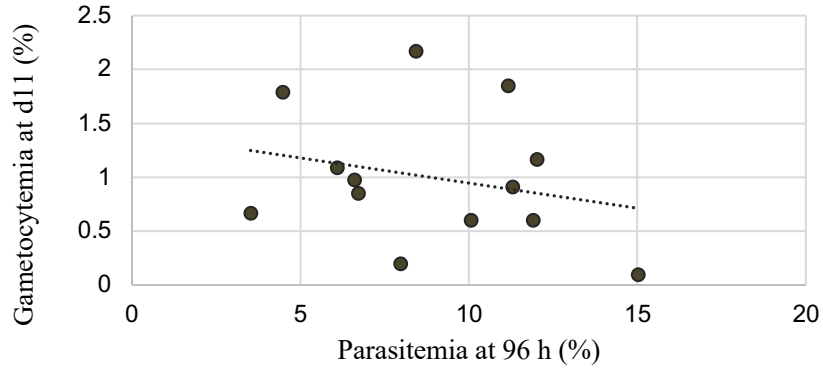


Figure S1: Anticorrelated relationship between parasitaemia and gametocytaemia

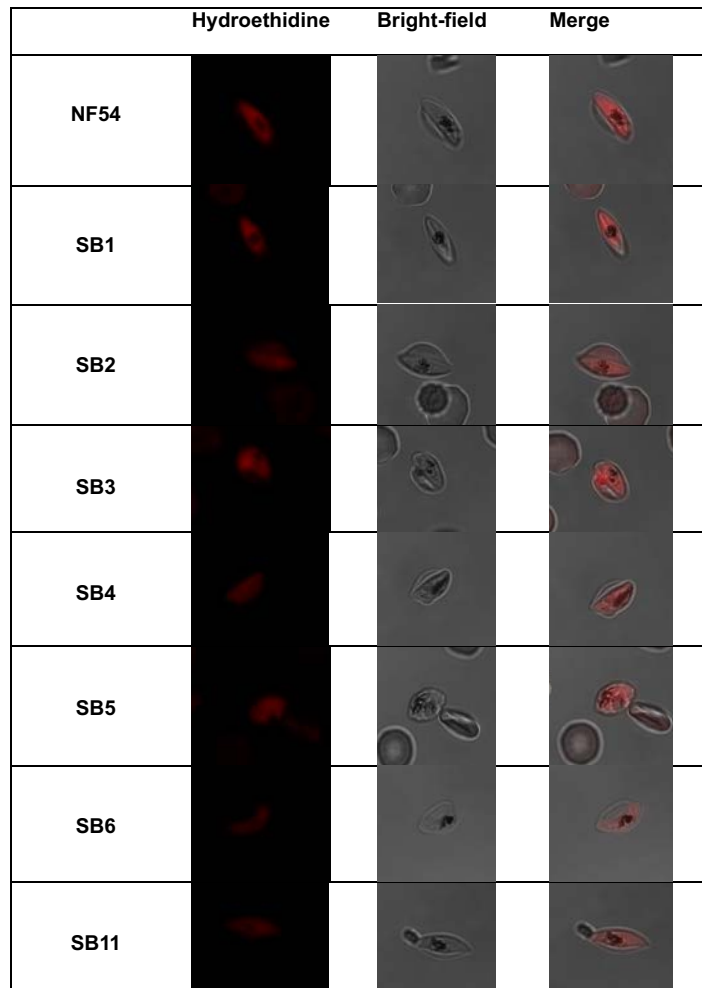


Figure S2: Confocal images of late-stage gametocytes from the isolates, stained with hydroethidine as a proxy of viability 100x magnification used.

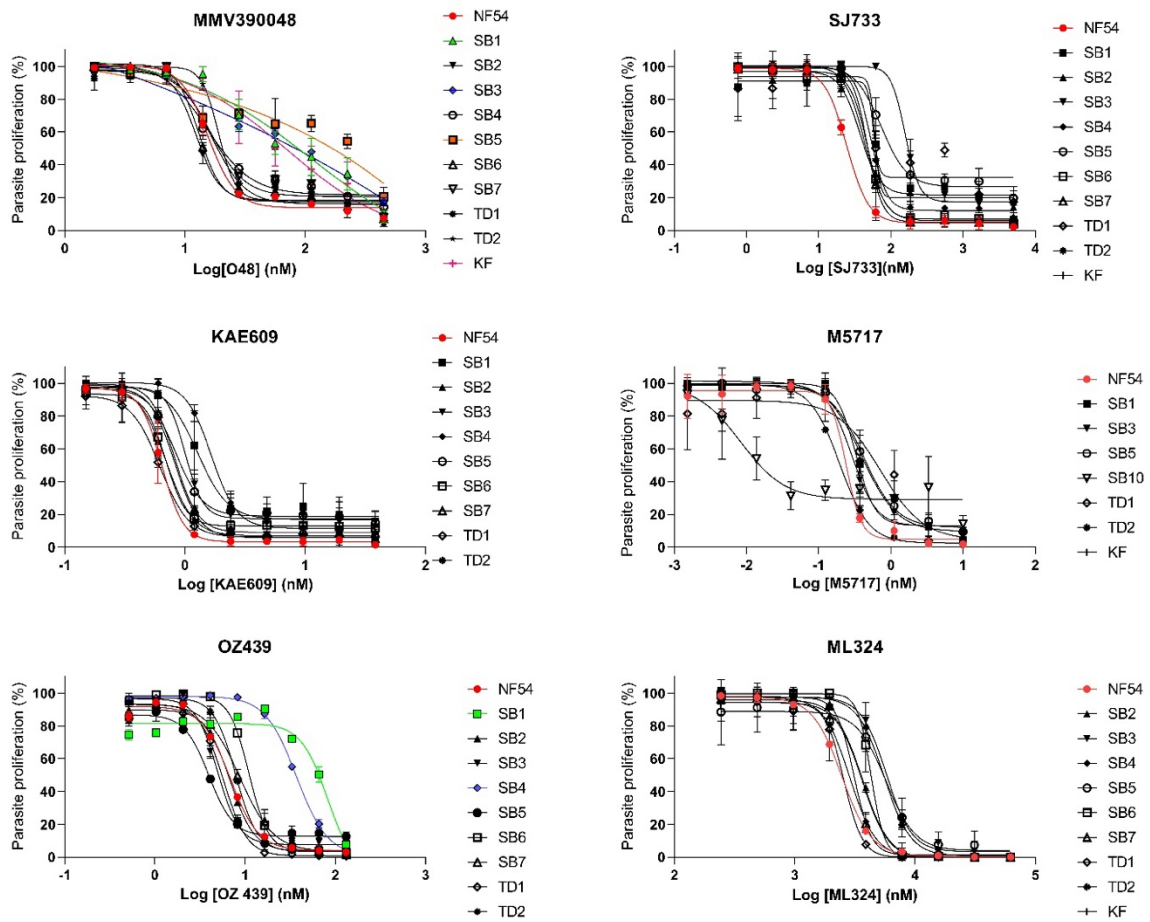


Figure S3: IC₅₀ curves of ABS parasites from the isolates compared to NF54 (in red) treated with the different compounds.

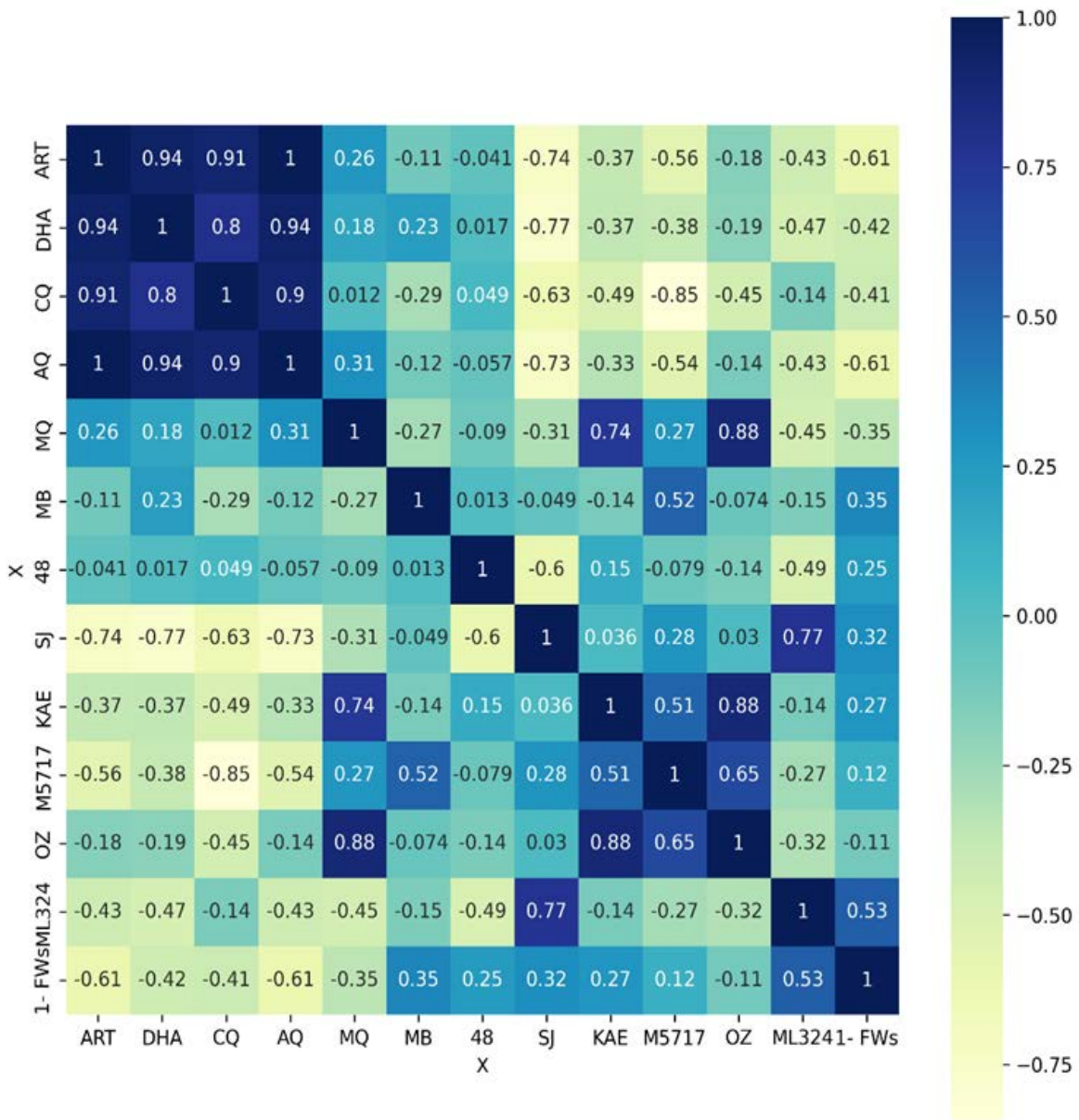


Figure S4: Correlation of drug efficacy between isolates across all isolates, for known antimalarial candidates as well as lead antimalarials.

2 Supplementary table

Table S2: IC₅₀ values of known antimalarial compounds against ABS parasites from PfNF54 and selected clinical isolates.

IC ₅₀ values (nM; avg ± S.E.)						
	ART	DHA	CQ	AQ	MQ	MB
NF54	6.5	2.1	6.8	3.0	20.0	21.9
SB4	0.38 ± 0.28	1	2.32	0.79 ± 0.12	85.78 ± 5.90	1.33 ± 0.33
SB5	3.14 ± 0.21	1	3.93 ± 0.07	2.34 ± 0.17	3.69 ± 0.32	1.33 ± 0.3
SB7	3.16 ± 0.40	1.25 ± 0.47	3.48 ± 0.16	2.09 ± 0.03	7.37 ± 0.55	63.31 ± 11.37
TD1	6.23 ± 0.25	2.38 ± 0.18	5.46 ± 0.31	4.49 ± 0.04	10.04 ± 1.21	3.67 ± 0.13
KF	3.75 ± 0.05	2.16 ± 0.01	ND	4.83 ± 0.44	4.6 ± 2.36	1

ND = not determined

IC₅₀ values are from three independent biological repeats, each performed in technical triplicates, average

Artemisinin (ART), dihydroartemisinin (DHA), chloroquine (CHQ), amodiaquine (ADQ), mefloquine (MQ) and methylene Blue (MB)

Table S2: IC₅₀ values of lead antimalarial candidate compounds against ABS parasites from PfNF54 and clinical isolates.

IC ₅₀ values (nM; avg ± S.E.)						
	MMV390048	SJ733	KAE609	M5717	OZ439	ML324 ^a
NF54	15.57 ± 1.10	25.08 ± 0.60	0.65 ± 0.05	0.25 ± 0.02	6.84 ± 0.15	2.47 ± 0.03
SB1	36.35 ± 1.55	61.28 ± 0.47	1.26 ± 0.19	0.43 ± 0.12	75.4 ± 7.34	2.61 ± 0.01
SB2	12.47 ± 0.53	37.51 ± 3.60	0.70 ± 0.05	ND	6.84 ± 0.20	3.65 ± 0.02
SB3	ND	170.33 ± 6.84	1.00 ± 0.04	0.28 ± 0.05	4.94 ± 0.09	5.81 ± 0.08
SB4	15.32 ± 1.01	52.82 ± 7.45	1.66	ND	37.51 ± 2.94	2.92 ± 0.16
SB5	ND	77.16 ± 12.33	0.72 ± 0.19	0.42 ± 0.05	3.96 ± 0.29	5.66 ± 0.38
SB6	13.07 ± 0.18	47.9 ± 10.38	0.68 ± 0.04	ND	10.90 ± 0.91	4.16 ± 0.14
SB7	17.17 ± 1.56	50.99 ± 4.98	0.82 ± 0.04	ND	8.83 ± 0.54	3.22 ± 0.24
SB10	ND	ND	0.45 ± 0.09	0.02 ± 0.01	ND	ND
TD1	19.02 ± 5.06	55.12 ± 1.86	0.61 ± 0.02	0.71 ± 0.21	5.01 ± 0.22	2.81 ± 0.43
TD2	18.96 ± 0.80	43.45 ± 2.99	0.82 ± 0.02	0.19 ± 0.01	7.83 ± 0.07	5.51 ± 0.09
KF	32.75 ± 0.82	42.08 ± 3.67	ND	0.32 ± 0.03	ND	3.81 ± 0.66

^a Data for ML324 is reported in μM

ND = not determined

IC₅₀ values are from three independent biological repeats, each performed in technical triplicates, average with S.E.

Table S3: Activity of frontrunner antimalarials against late-stage PfNF54 gametocytes determined using pLDH assay, compared to published values.

Compound	IC ₅₀ ± S.E. (nM)	Published IC ₅₀	Reference
MMV390048	230.70 ± 37.37	214 nM (stage II/III)	(Paquet et al., 2017)
SJ733	1.11 ± 0.49	5 mg/kg (ED ₅₀ in <i>P. berghei</i>) ^a	(Dechering et al., 2017)
KAE609	1.08 ± 0.38	100 (reducing transmission at 5 μM)	(van Pelt-Koops et al., 2012)
M5717	3.10 ± 0.34	2 nM (stage IV)	(Baragaña et al., 2015, Plouffe et al., 2016)
OZ439	3.62 ± 0.41	2 nM (stage II/III)	(Giannangelo et al., 2020)
ML324	70.37 ± 3.80	77 nM (stage V)	(Reader et al., 2021)

^a No *P. falciparum* data available

Table S4: Microsatellite markers used in study with the unique number of alleles identified for each isolate's MOI.

Isolate	Ara2	AS1	AS11	AS12	AS14	AS15	AS19	AS2	AS21	AS25	AS3	AS31	AS32
SB1	0	0	1	0	1	1	0	1	1	0	0	1	1
SB2	0	0	1	0	1	1	0	1	0	0	1	0	1
SB3	2	1	2	2	1	2	2	1	0	4	1	2	1
SB4	2	1	1	2	2	2	2	1	0	1	2	2	1
SB5	3	1	1	2	4	2	1	2	0	3	1	2	3
SB6	1	1	1	1	1	1	1	1	0	1	1	1	1
SB7	2	1	2	2	2	2	2	3	2	6	3	3	1
TD1	1	1	1	1	1	1	1	1	0	1	1	1	1
TD2	1	1	1	1	1	1	1	1	0	1	1	1	1
KF	3	2	4	3	6	2	3	2	2	2	3	4	2

Isolate	AS34	AS8	B7M19	PFG377	TA109	TA40	TA60	TA81	TA87	AS7	PolyA	PfPK2
SB1	1	1	0	1	0	0	1	0	0	1	2	0
SB2	1	1	0	1	0	0	1	0	0	1	1	0
SB3	2	2	1	1	2	1	1	1	1	1	1	0
SB4	1	1	1	1	1	1	3	2	2	2	1	0
SB5	1	2	2	1	2	2	1	3	1	2	3	0
SB6	1	1	0	0	1	1	1	1	1	1	1	1
SB7	1	3	2	2	3	2	2	2	1	1	2	0
TD1	1	1	1	1	1	1	1	1	2	1	2	0
TD2	1	1	1	1	1	1	1	1	1	1	2	1
KF	1	1	1	3	3	5	2	4	2	1	1	0

Table S5: F_{WS} indices for the clinical isolates.

Isolate	SB1	SB2	SB3	SB4	SB5	SB6	SB7	TD1	TD2	KF
F_{WS}	0.959	1	0.690	0.672	0.533	1	0.413	0.953	0.977	0.302

* F_{WS} was calculated for each sample using equation $F_{WS} = 1 - Hw/Hs$, where Hw is the allele frequency of each unique allele found at a particular locus for each individual and Hs is the heterozygosity of the local parasite population.