SUPPORTING INFORMATION

Plasmodium falciparum Cyclic Amine Resistance Locus, PfCARL: a Resistance Mechanism for Two Distinct Compound Classes

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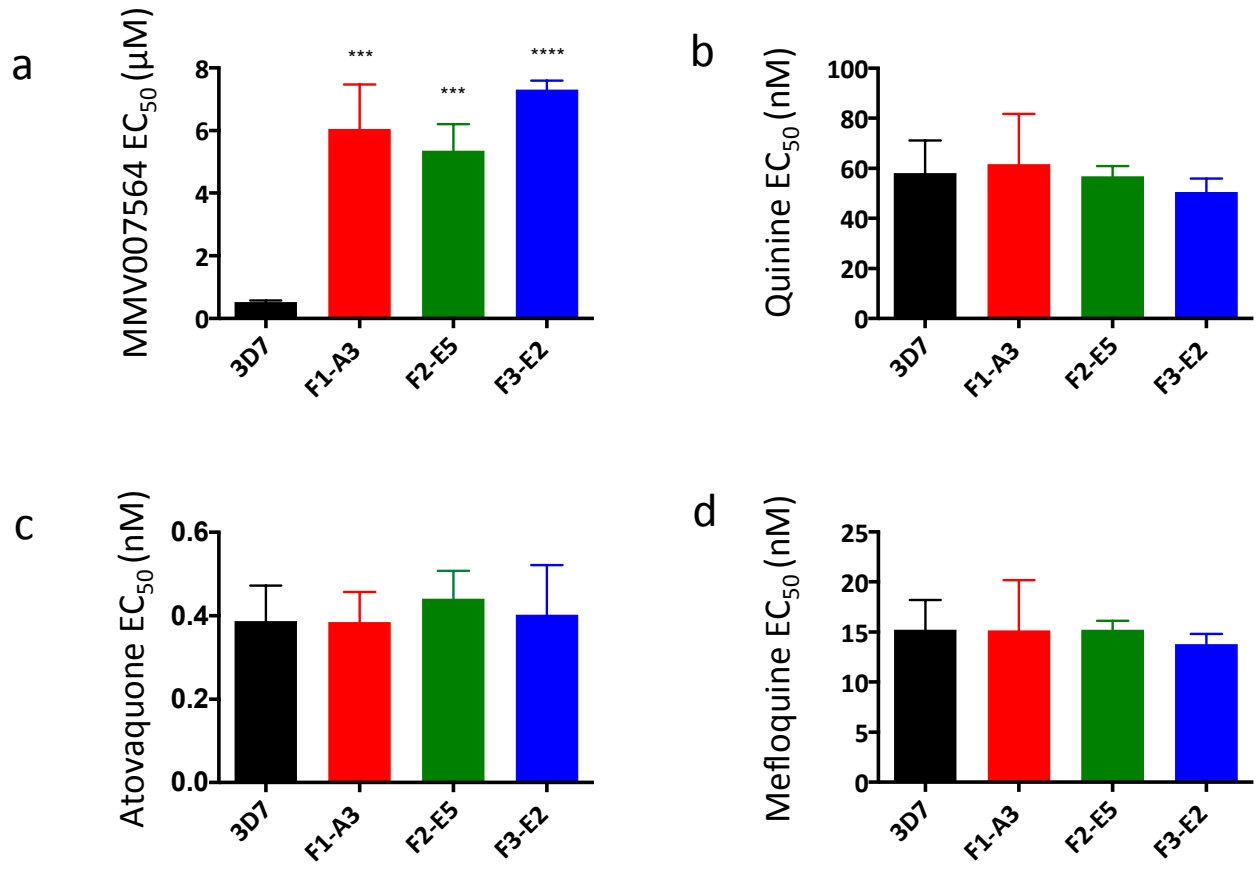


Figure S1. EC_{50}s of 3 clones from 3 independent selections showing at least a statistically significant 10-fold increase for MMV007564 (a) compared to the 3D7 parent but not for other antimalarials, quinine (b), atovaquone (c) and mefloquine (d). Bars are means of 3 independent experiments showing standard deviations. Statistical analyses were performed using ordinary one-way ANOVA followed by Dunnett's multiple comparisons test.

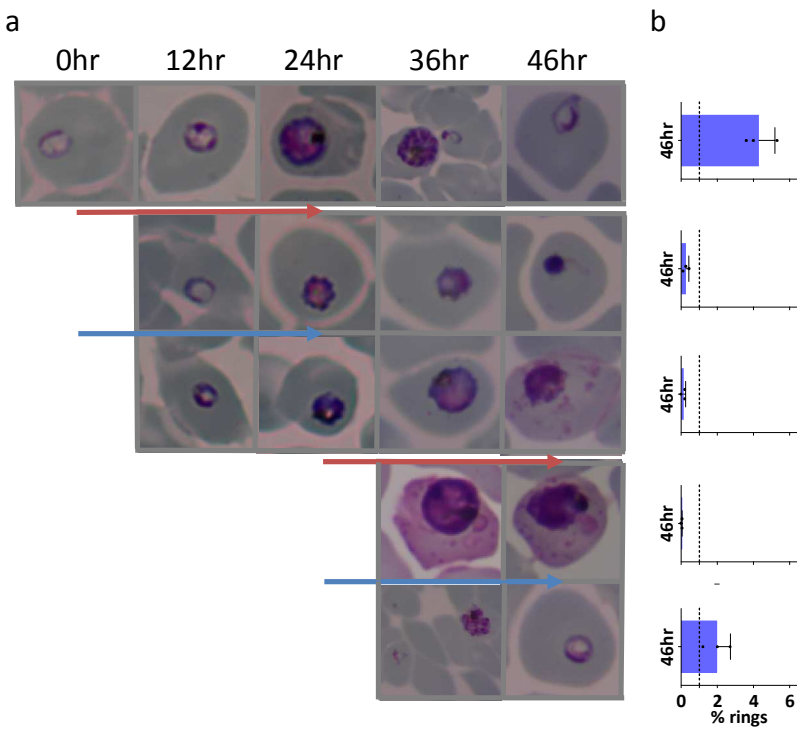
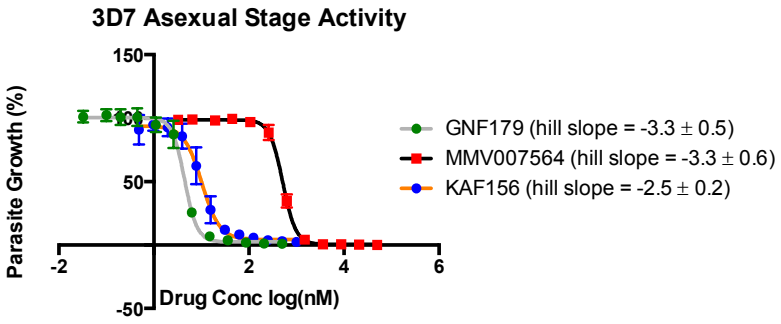


Figure S2. (a) Susceptibility of the different asexual blood stages to 10xEC_{50} of MMV007564 (red arrow) and GNF179 (blue arrow) at different time points starting from tightly synchronized, 0-5hr old rings. Top panel is no compound. Arrows indicate the period when each compound was present in the culture (0-24hr and 24-46hr). (b) Bars indicate average ring-stage parasitemia at the 46hr time point and error bars indicate standard deviation for 3 independent experiments. Broken lines at 1% rings indicate starting parasitemia at 0hr.

a



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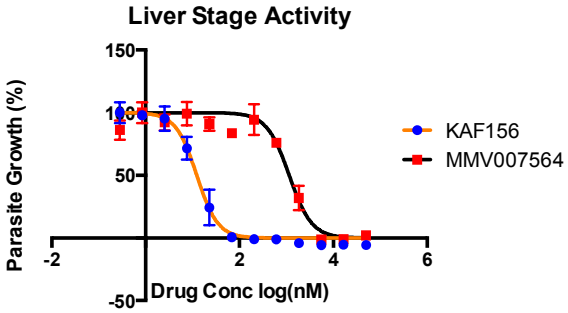

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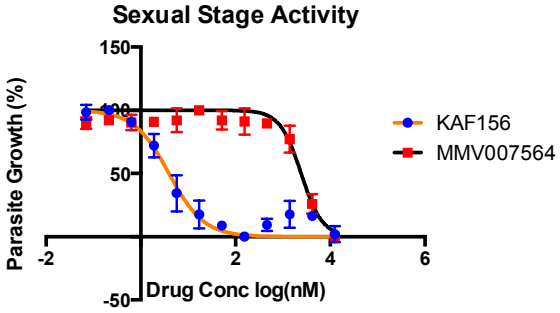

Figure S3. Dose response curves for compounds used for determining the timing of action (GNF179 and MMV007564) and multi-stage activity (KAF156 and MMV007564) at the (a) asexual stage of 3D7, (b) liver stage and (c) sexual stage. Average dose response curves for at least 3 independent experiments are shown except for the gametocyte stage with 2 independent experiments.

Table S1. Whole genome sequencing haplotypecaller sequencing results for Gen16, Gen28, and Gen50 (see attached excel sheet – TableS1_and_S3)

Table S2. Applied filters for GATK's HaplotypeCaller

SNV Filters		*INDEL Filters*	
Filter Name	*Filter Value*	*Filter Name*	*Filter Value*
ReadPosRankSum	> 8.0	*ReadPosRankSum*	< -20
	< -8.0	*QUAL*	< 500
QUAL	< 500	*QD*	< 2
QD	< 2	*DP*	< 7
MQRankSum	< -12.5		
DP	< 7		

Table S3. Summary lists of PfCARL allele mutations identified as conferring benzimidazolyl piperidine and imidazolopiperazine resistance (see attached excel sheet – TableS1_and_S3)

Table S4. Inactive benzimidazolyl piperidines with EC_{50} values > 3.5µM against the 3D7 parent.

Compound	*EC50 (nM) ± SD*			
	Parent 3D7	*MMV007564^R PfCARL: L1136P*	*MMV007564^R PfCARL: L833I*	*MMV007564^R PfCARL :L1073Q*
Benzimidazolyl piperidines with the methyl benzyl of MMV007564 replaced with various chemical groups				
1	11612 ± 1686	12375 ± 2279	12011 ± 3727	9367 ± 2483
2	4466 ± 638	4298 ± 1426	3877 ± 1103	3016 ± 920
3	54883 ± 8276	61270 ± 11831	50773 ± 7835	49963 ± 4311
Benzimidazolyl piperidines with the thiophen of MMV007564 replaced with various chemical groups				
7	11488 ± 1875	17323 ± 7007	17240 ± 4820	15420 ± 1051
10	9437 ± 2344	9338 ± 2900	9408 ± 1677	7976 ± 1536
11	20090 ± 5409	21447 ± 6509	22180 ± 4132	17120 ± 3304
12	8522 ± 1741	7680 ± 2224	8490 ± 3015	6510 ± 1826
13	5714 ± 997	5520 ± 750	6047 ± 272	5648 ± 147
14	27917 ± 3530	27567 ± 4755	25710 ± 4692	23620 ± 2379