

Supporting Information

Antimalarial mechanism of action of the natural product 9-methoxystrobilurin G

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Table S1. Summary statistics of genome sequencing data.

Parasite	Raw reads	¹ Pre-processed reads	² Aligned Reads	³ Coverage	⁴ SNP (all/PASS)	⁴ Indel (all/PASS)
3D7 parent	9,729,178	9,617,168	9,159,246	59x	140/90	390/387
3D7_1_G5	8,217,770	8,176,074	7,774,453	50x	84/57	206/206
3D7_2_B6	7,722,548	7,675,696	7,154,328	46x	130/103	275/275
3D7_3_E6	8,111,514	8,066,894	6,699,813	43x	65/37	177/177
Dd2 parent	9,176,950	9,175,230	7,016,891	46x	365/193	640/404
Dd2_4_G5	8,646,382	8,621,016	8,137,017	54x	162/93	499/318
Dd2_1_F3	9,126,664	9,100,263	9,001,302	60x	286/190	942/627

¹Preprocessed reads remaining after fastp filtering.

²Reads aligned to reference genome.

³Coverage was calculated from the total bases sequenced in aligned reads and the genome size.

⁴Variants called by GATK.

Table S2. Copy number variants (CNVs) among 9MG-resistant parasite clones.

Genome sequencing data from the 3D7 and Dd2 parents and 9MG-resistant clonal lines of *P. falciparum* from independent selection experiments were analysed using the cn.mops tool. No CNVs were detected in Dd2 or clonal lines from this parent. Twelve CNVs were reported among 3D7 and clonal lines from this parent (3D7_1_G5, 3D7_2_B6, and 3D7_3_E6). The copy numbers in each parasite are indicated in columns 5–8.

chromosome	start	end	width (bp)	Parasite line			
				3D7	2_B6	3_E6	1_G5
Pf3D7_01_v3	555001	575000	20000	2	1	1	1
Pf3D7_02_v3	815001	830000	15000	2	1	1	1
Pf3D7_03_v3	1	80000	80000	8	0	0	0
Pf3D7_04_v3	960001	975000	15000	1	1	1	2
Pf3D7_06_v3	465001	480000	15000	2	1	1	1
Pf3D7_07_v3	1075001	1090000	15000	2	1	1	1
Pf3D7_09_v3	1380001	1400000	20000	4	1	1	1
Pf3D7_11_v3	125001	145000	20000	2	1	1	1

Pf3D7_11_v3	1935001	1950000	15000	2	1	1	1
Pf3D7_11_v3	1985001	2000000	15000	2	1	1	1
Pf3D7_13_v3	2910001	2925236	15236	1	0	1	1
Pf3D7_14_v3	555001	570000	15000	2	1	1	1

Table S3. Indel variants in protein coding genes among 9MG-resistant clones.

Gene ID	MFS ¹	MIS ²	9MG-resistant clone				
			3D7_1_ G5	3D7_2_ B6	3D7_3_ E6	Dd2_1_ F3	Dd2_4_ G5
PF3D7_1244400	-2.897	0.149	NNYNN K480K	NNYNN K480K	NNYNN K480K	No change	No change
PF3D7_1118500	-2.956	0.123	D505R?	D505R?	D505R?	No change	No change
PF3D7_1348400	-2.777	0.156	No change	No change	No change	N507_D5 08insVPIN	N507_D50 8insVPIN
PF3D7_1229600	-2.449	0.462	No change	No change	No change	K31fs	K31fs

¹Gene mutagenesis fitness score.

²Gene mutagenesis index score.

Table S4. Predicted free energies of ligands binding to *P. falciparum* CYTB receptors.

CYTB	¹ 9MG	² ATQ
Wild-type	-8.1	-9.6
Y126C	-7.8	-9.5
G131S	-7.8	-9.6
V259L	-7.7	-9.7
F267C	-7.6	-9.1

¹Top-ranked pose 9-methoxytribilurin G (kcal/mol) reported by Autodock Vina 1.2.0.

²Second top-ranked pose Atovaquone (kcal/mol) reported by Autodock Vina 1.2.0.

Table S5. 9MG target proteins with significant abundance ratios from affinity pull-down.

Gene ID	Log2-Re	Log2-Rc	Product Description	GeneSymbol	MW (kDa)
PF3D7_062260 0	6.643856	2.664837	cytochrome b-c1 complex subunit 9, putative	QCR9	11.7
PF3D7_143500 0	6.643856	1.168779	cytochrome c oxidase assembly protein COX15, putative	COX15	50.4

PF3D7_101230 0	6.643856	1.016332	cytochrome b-c1 complex subunit 7, putative	QCR7	23.0
PF3D7_140940 0	5.601859	4.425695	conserved protein, unknown function	N/A	30.5
PF3D7_041720 0	5.479393	4.401933	bifunctional dihydrofolate reductase-thymidylate synthase	DHFR-TS	71.7
PF3D7_031280 0	4.014295	3.432586	60S ribosomal protein L26, putative	N/A	14.8
PF3D7_082290 0	4.334338	3.39004	PhIL1-interacting candidate PIC2	PIC2	138
PF3D7_121140 0	6.643856	3.308097	heat shock protein DNAJ homologue Pfj4	PfJ4	28.1
PF3D7_122340 0	4.508444	3.293279	phospholipid-transporting ATPase, putative	N/A	190
PF3D7_060870 0	2.192045	2.94421	T-complex protein 1 subunit zeta	CCT6	61.5
PF3D7_120620 0	2.537345	2.906179	eukaryotic translation initiation factor 3 subunit C, putative	EIF3C	116
PF3D7_030550 0	4.419997	2.896421	protein dopey homolog, putative	N/A	468
PF3D7_010630 0	2.745472	2.801665	calcium-transporting ATPase	ATP6	139
PF3D7_146630 0	2.323339	2.771092	26S proteasome regulatory subunit RPN2, putative	RPN2	133
PF3D7_061800 0	6.75884	2.714107	conserved Plasmodium membrane protein, unknown function	N/A	92.8
PF3D7_082100 0	4.474433	2.698633	conserved Plasmodium protein, unknown function	N/A	60.5
PF3D7_135250 0	4.908529	2.685306	thioredoxin-related protein, putative	N/A	24.08
PF3D7_110860 0	2.526357	2.674541	endoplasmic reticulum- resident calcium binding protein	ERC	39.4
PF3D7_040540 0	2.382838	2.507735	pre-mRNA-processing- splicing factor 8, putative	PRPF8	366
PF3D7_134260 0	2.295946	2.414185	myosin A	MyoA	92.3

PF3D7_051260 0	2.04412	2.411633	ras-related protein Rab-1B	RAB1b	22.9
PF3D7_144790 0	2.12688	2.343524	multidrug resistance protein 2	MDR2	119
PF3D7_141070 0	4.6932	2.33219	conserved protein, unknown function	N/A	39.8
PF3D7_101690 0	2.055426	2.32854	early transcribed membrane protein 10.3	ETRAMP10.3	12.0
PF3D7_030640 0	5.000926	2.316118	FAD-dependent glycerol-3-phosphate dehydrogenase, putative	N/A	74.7
PF3D7_135390 0	2.501206	2.306833	proteasome subunit alpha type-7, putative	N/A	27.2
PF3D7_135320 0	2.300332	2.276241	membrane associated histidine-rich protein 2	MAHRP2	15.8
PF3D7_052300 0	3.744632	2.264318	multidrug resistance protein 1	MDR1	162
PF3D7_072040 0	2.356379	2.241959	apoptosis-inducing factor, putative	AIF	72.7
PF3D7_082440 0	2.531873	2.207867	nucleoside transporter 2	NT2	67.6
PF3D7_092260 0	3.713577	2.131367	glutamine synthetase	N/A	63.2
PF3D7_070250 0	5.777655	2.076488	Plasmodium exported protein, unknown function	N/A	27.7
PF3D7_080180 0	2.676273	2.064931	mannose-6-phosphate isomerase, putative	N/A	117
PF3D7_091910 0	3.150463	2.063572	DnaJ protein, putative	N/A	43.3
PF3D7_114250 0	2.470842	2.023057	60S ribosomal protein L28	RPL28	14.4
PF3D7_031250 0	6.643856	2.016601	multidrug-resistant modulator MFR3	MFR3	67.8
PF3D7_091420 0	6.643856	1.968535	phospholipid or glycerol acyltransferase, putative	N/A	49.3
PF3D7_021400 0	2.882198	1.962697	T-complex protein 1 subunit theta	CCT8	61.0

PF3D7_130620 0	4.547325	1.923888	conserved protein, unknown function	N/A	56.8
PF3D7_141390 0	6.643856	1.920439	DnaJ protein, putative	N/A	45.2
PF3D7_136180 0	3.267003	1.912002	glideosome-associated connector	GAC	291
PF3D7_141920 0	5.465976	1.906033	thioredoxin-like protein, putative	ATrx1	80.2
PF3D7_145700 0	4.622241	1.899937	signal peptide peptidase	SPP	47.6
PF3D7_072780 0	4.395658	1.877485	cation transporting ATPase, putative	N/A	22.5
PF3D7_145010 0	3.438976	1.872395	signal recognition particle subunit SRP54	SRP54	56.0
PF3D7_133830 0	4.609773	1.865624	elongation factor 1-gamma, putative	EF-1gamma	47.8
PF3D7_090490 0	2.544133	1.853713	copper-transporting ATPase	CuTP	299
PF3D7_091540 0	3.873423	1.845214	ATP-dependent 6- phosphofructokinase	PFK9	159
PF3D7_083170 0	2.518524	1.836483	heat shock protein 70	HSP70x	75.0
PF3D7_050100 0	2.123834	1.812537	Plasmodium exported protein, unknown function	N/A	30.7
PF3D7_141610 0	2.820481	1.805644	protein SEY1, putative	SEY1	110
PF3D7_052750 0	2.015757	1.786886	Hsc70-interacting protein	HIP	51.1
PF3D7_143810 0	3.197067	1.783303	translocation protein SEC62, putative	SEC62	43.9
PF3D7_102960 0	2.515876	1.722246	adenosine deaminase	ADA	42.5
PF3D7_112900 0	4.408059	1.708941	spermidine synthase	SPDS	36.6
PF3D7_135140 0	2.520006	1.706825	60S ribosomal protein L17, putative	N/A	23.4

PF3D7_142370 0	2.157608	1.702477	NTF2-like domain-containing protein, putative	N/A	183
PF3D7_030290 0	4.212225	1.682469	exportin-1, putative	N/A	148
PF3D7_134480 0	3.833956	1.61589	aspartate carbamoyltransferase	ATCase	43.3
PF3D7_144230 0	6.643856	1.612855	tRNA import protein tRIP	tRIP	46.4
PF3D7_061680 0	3.686327	1.58392	malate:quinone oxidoreductase	MQO	59.5
PF3D7_102140 0	4.126029	1.561551	endomembrane protein 70, putative	N/A	72.5
PF3D7_060450 0	2.354691	1.554894	GYF domain-containing protein, putative	N/A	334
PF3D7_051570 0	3.413757	1.551349	glideosome-associated protein 40	GAP40	51.8
PF3D7_133130 0	3.07946	1.510493	signal peptidase complex catalytic subunit SEC11	SEC11	21.1
PF3D7_061210 0	2.36085	1.470113	eukaryotic translation initiation factor 3 subunit L, putative	EIF3L	78.129
PF3D7_123560 0	2.223538	1.457757	serine hydroxymethyltransferase	SHMT	49.8
PF3D7_136270 0	2.446947	1.452309	conserved Plasmodium protein, unknown function	N/A	242
PF3D7_070970 0	2.39187	1.449337	prodrug activation and resistance esterase	PARE	42.4
PF3D7_053230 0	2.482806	1.440916	Plasmodium exported protein (PHISTb), unknown function	N/A	60.2
PF3D7_145680 0	2.047686	1.437117	V-type H(+)-translocating pyrophosphatase, putative	VP1	76.4
PF3D7_111670 0	2.057354	1.408477	dipeptidyl aminopeptidase 1	DPAP1	80.4
PF3D7_140560 0	6.643856	1.398309	ribonucleoside-diphosphate reductase small chain, putative	RNR	40.6
PF3D7_081120 0	2.22267	1.379769	ER membrane protein complex subunit 1, putative	EMC1	134

PF3D7_120430 0	3.976124	1.358605	eukaryotic translation initiation factor 5A	EIF5A	17.6
PF3D7_111730 0	3.531225	1.348071	conserved protein, unknown function	N/A	15.3
PF3D7_100880 0	2.062661	1.335456	nucleolar protein 5, putative	NOP5	53.4
PF3D7_091040 0	3.326237	1.330041	selenide water dikinase, putative	N/A	140
PF3D7_121620 0	2.397081	1.329268	glycerol-3-phosphate dehydrogenase [NAD(+)], putative	N/A	41.2
PF3D7_052400 0	3.002223	1.305399	karyopherin beta	KASbeta	127
PF3D7_142980 0	2.209088	1.290911	coatomer subunit beta, putative	N/A	160
PF3D7_123880 0	4.005243	1.276177	acyl-CoA synthetase	ACS11	92.1
PF3D7_147120 0	4.803027	1.27171	inorganic anion exchanger, inorganic anion antiporter	SulP	75.5
PF3D7_122310 0	2.495274	1.26787	cAMP-dependent protein kinase regulatory subunit	PKAr	50.8
PF3D7_041430 0	5.418259	1.247838	Rab5-interacting protein, putative	N/A	13.2
PF3D7_040560 0	2.876851	1.246448	TMEM33 domain-containing protein, putative	N/A	37.8
PF3D7_093280 0	3.3612	1.232008	importin alpha re-exporter, putative	CSE1	159
PF3D7_146190 0	3.155645	1.229964	valine--tRNA ligase, putative	N/A	128
PF3D7_080200 0	2.65922	1.227006	glutamate dehydrogenase, putative	GDH3	160
PF3D7_101470 0	6.643856	1.214198	prohibitin 2	PHB2	34.8
PF3D7_124280 0	2.011894	1.205719	rab specific GDP dissociation inhibitor	rabGDI	52.3
PF3D7_146870 0	2.356969	1.205527	eukaryotic initiation factor 4A	eIF4A	45.3

PF3D7_110580 0	3.88338	1.198138	conserved Apicomplexan protein, unknown function	N/A	30.7
PF3D7_132510 0	2.101956	1.188305	phosphoribosylpyrophosphate synthetase	N/A	49.4
PF3D7_110570 0	2.343411	1.180779	tRNA-splicing ligase RtcB, putative	RTCB	56.3
PF3D7_141250 0	6.643856	1.170925	actin II	ACT2	42.6
PF3D7_121200 0	6.643856	1.165314	glutathione peroxidase-like thioredoxin peroxidase	TPx(Gl)	24.0
PF3D7_143980 0	2.246447	1.164797	vesicle-associated membrane protein, putative	N/A	27.7
PF3D7_020470 0	3.681436	1.157234	hexose transporter	HT1	56.4
PF3D7_132340 0	2.767892	1.138535	60S ribosomal protein L23	RPL23	22.1
PF3D7_042460 0	2.831391	1.134971	Plasmodium exported protein (PHISTb)	N/A	36.0
PF3D7_070740 0	3.220294	1.108446	ATPase family AAA domain-containing protein 3A, putative	ATAD3A	78.2
PF3D7_081060 0	2.056556	1.105652	ATP-dependent RNA helicase DBP1, putative	DBP1	108
PF3D7_135780 0	2.097063	1.09305	T-complex protein 1 subunit delta	CCT4	58.0
PF3D7_133810 0	2.33467	1.057231	26S proteasome regulatory subunit RPN3, putative	RPN3	59.1
PF3D7_123040 0	6.643856	1.037119	ATP-dependent protease subunit ClpQ	ClpQ	22.9
PF3D7_051360 0	3.183048	1.032676	deoxyribodipyrimidine photolyase, putative	N/A	129
PF3D7_102190 0	2.565913	1.029863	PHAX domain-containing protein, putative	N/A	267
PF3D7_120100 0	2.068073	1.024507	Plasmodium exported protein (PHISTb), unknown function	N/A	77.1
PF3D7_121270 0	2.144606	1.019925	eukaryotic translation initiation factor 3 subunit A, putative	EIF3A	166

PF3D7_100400 0	2.111574	1.019904	60S ribosomal protein L13, putative	N/A	23.8
PF3D7_101290 0	2.20428	1.018212	autophagy-related protein 18	ATG18	43.5
PF3D7_147110 0	2.691135	1.016749	exported protein 2	EXP2	33.4
PF3D7_135450 0	6.643856	1.011391	adenylosuccinate synthetase	ADSS	50.0

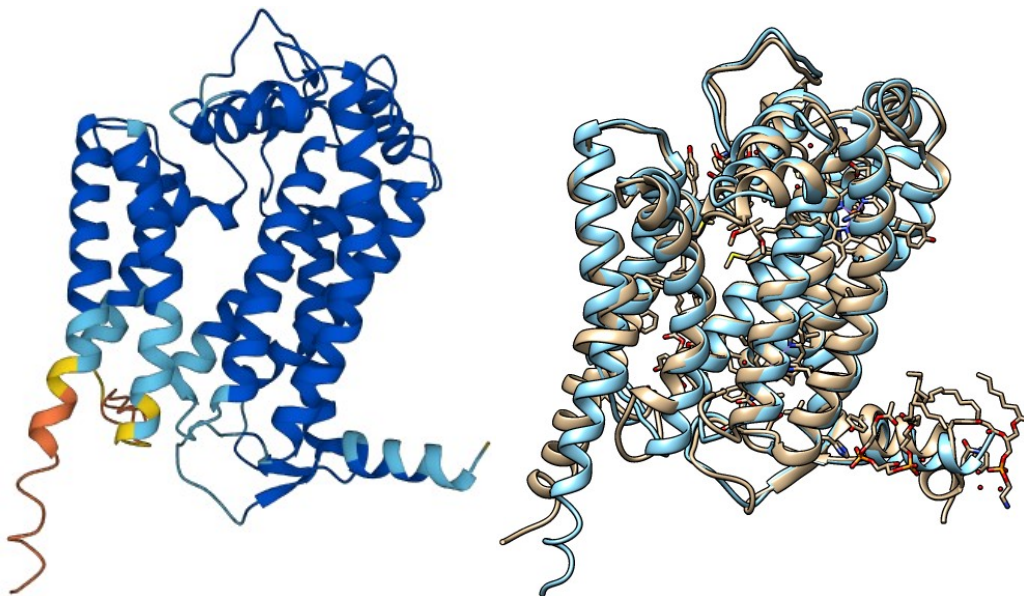


Figure S1. *Ab initio* structure of wild-type *Plasmodium falciparum* cytochrome b (PfCYTB).

The AlphaFold v2.0 *ab initio* structure determined for PfCYTB (Uniprot: Q7HP03) is shown on the left. Residues are colored according to model confidence: dark blue, very high (pLDDT per residue confidence score > 90); cyan, confident (90 > pLDDT > 70); yellow, low (70 > pLDDT > 50), and red, very low (pLDDT < 50). On the right, the *ab initio* PfCYTB structure (cyan) was superposed with

Saccharomyces cerevisiae yeast CYTB in the yeast bc1 complex X-ray structure solved to 1.9 Angstrom resolution (PDB: 3CX5; chain C CYTB protein in brown, other proteins in the complex have been removed for clarity).

Figure S2. HRMS (ESI) and 1H NMR spectra for compound 9M-P.

