Supplementary data

Identifying Hexahydroquinolines as New Antimalarial Candidates with Potent Blood Stage and Transmission-Blocking Activity

Manu Vanaerschot[1], Leonardo Lucantoni[2], Tao Li[3], Jill M. Combrinck[4], Andrea Ruecker[5,$], T.R. Santha Kumar[1], Kelly Rubiano[1], Pedro E. Ferreira[6], Giulia Siciliano[7], Sonia Gulati[1], Philipp P. Henrich[1], Caroline L. Ng[1], James M. Murithi[1], Victoria C. Corey[8], Sandra Duffy[2], Ori J. Lieberman[1], M. Isabel Veiga[6], Robert E. Sinden[5], Pietro Alano[7], Michael J. Delves[5], Kim Lee Sim[3], Elizabeth A. Winzeler[8], Timothy J. Egan[9], Stephen L. Hoffman[3], Vicky M. Avery[2], David A. Fidock[1,10,*]

[1]Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY, USA; [2]Discovery Biology, Griffith Institute for Drug Discovery, Griffith University, Nathan, Queensland, Australia; [3]Sanaria Inc., Rockville, MD, USA; [4]Division of Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa; [5]Department of Life Sciences, Imperial College, London, UK; [6]Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; [7]Dipartimento di Malattie Infettive, Parassitarie ed Immunomediate, Istituto Superiore di Sanità, Rome, Italy; [8]University of California, San Diego, School of Medicine, La Jolla, CA, USA; [9]Department of Chemistry, University of Cape Town, Cape Town, South Africa; [10]Division of Infectious Diseases, Department of Medicine, Columbia University Medical Center, New York, NY, USA.

*Corresponding author: df2260@cumc.columbia.edu

[$]Current address: Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400, Thailand; Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7FZ, UK

Table of contents

Supplementary Table 1. Gametocytocidal activity of the 149 resourced selected screening hits.

				Luciferase early		Imaging late GAM	
	Compound	**Commercial source**	**SMILES**	**GAM IC_{50} (nM)**	**Early GAM pIC_{50}**	**IC_{50} (nM)**	**Late GAM pIC_{50}**
1	GNF-Pf-1319		CCc4ccc3NC(=O)C1(NN=C(S1)c2ccc(C)cc2)c3c4	> 5000	<5.40	955.0	6.0
2	GNF-Pf-4550		CCOC(=O)C2Nc1ccc(C)cc1C=2/N=N/N(C)C	> 5000	<5.40	> 5000	<5.40
3	GNF-Pf-5036		C/C(=N\NC(=O)c1ccc(C)cc1)/c2ccccn2	> 5000	<5.40	461.0	6.3
4	GNF-Pf-771		CN4C(=O)C(Cl)=C1c3c(C(=O)c2ccccc12)c(Cl)ccc34	5.6	8.3	4.7	8.3
5	GNF-Pf-5134		CCN2c1ccccc1N(C)C2(C)c3ccccc3	> 5000	<5.40	1542.0	5.8
6	MMV666596, GNF-Pf-366		CSC3=N/C(=N\c1c(C)cccc1C)/C2(CCC(CC2)C(C)(C)C)N3c4ccc(Cl)cc4	> 5000	<5.40	> 5000	<5.40
7	GNF-Pf-3542		[O-][N+](=O)C1=CC=C(O1)C(=O)Nc3ccc2OC(=Nc2c3)c4ccccc4F	> 5000	<5.40	802.0	6.1
8	GNF-Pf-1329		Cc1ccc(cc1Br)C3=Nc2cc(ccc2O3)NC(=O)C4=CC=C(O4)[N+]([O-])=O	> 5000	<5.40	971.0	6.0
9	GNF-Pf-2740		[O-][N+](=O)C1=CC=C(O1)C(=O)Nc3ccc2OC(=Nc2c3)c5cccc4ccccc45	4362.7	5.4	1830.0	5.7
10	GNF-Pf-783, TCMDC-123925	ChemDiv	Cc1cccc(c1)C3=Nc2cc(ccc2O3)NC(=O)C4=CC=C(O4)[N+]([O-])=O	> 5000	<5.40	352.0	6.5
11	GNF-Pf-4107, SJ000011260	ChemDiv	Cc1cccc(c1)C(=O)NCC2=NNC(=S)N2c3cccc(c3)C(F)(F)F	> 5000	<5.40	> 5000	<5.40
12	GNF-Pf-4479	Vitas-M Laboratory, Ltd	CC3NC1CC(CC(=O)C=1C(c2ccc(Cl)cc2)C=3C(=O)OC4CCCC4)c5ccc(Cl)cc5	287.2	6.5	1311.0	5.9
13	GNF-Pf-5592		CCCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3cccc(Cl)c3Cl)c4ccc(cc4)OC	499.5	6.3	991.0	6.0
14	GNF-Pf-5274		COc1ccc(cc1)C2CC(=O)C4=C(C2)NC(C)=C(C(=O)OC3CCCC3)C4c5ccc(Cl)cc5	3727.0	5.4	> 5000	<5.40
15	GNF-Pf-5575	ChemDiv	COc1ccc(cc1)C2CC(=O)C4=C(C2)NC(C)=C(C(=O)OC3CCCC3)C4c5ccc(Cl)cc5Cl	359.2	6.4	1175.8	5.9
16	GNF-Pf-5138		Cc1cc(C)c3c(c1)C2C(=S)SSC=2C(C)(C)N3C(=O)COC5=Nc4ccccc4S5	> 5000	<5.40	1265.0	5.9
17	GNF-Pf-5547		COc1ccc(cc1)C2CC(=O)C4=C(C2)NC(C)=C(C(=O)OC3CCCC3)C4c5ccc(F)cc5	> 5000	<5.40	> 5000	<5.40
18	GNF-Pf-5209	ChemDiv	COc5cc4c(nc2c(C(Cc1ccccc1)=NN2c3ccccc3)c4cc5OC)c6cc(Br)ccc6O	242.6	6.6	791.2	6.1
19	GNF-Pf-5505		COc1ccc(cc1OC)C2CC(=O)C3=C(C2)NC(C)=C(C(=O)OC(C)C)C3c4ccc(F)cc4	225.7	6.6	> 5000	<5.40
20	**GNF-Pf-5640**	**ChemDiv**	**COc1ccc(cc1OC)C2CC(=O)C3=C(C2)NC(C)=C(C(=O)OC(C)C)C3c4ccccc4C**	**15.1**	**7.8**	**92.1**	**7.0**
21	GNF-Pf-5651		CCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccc(F)cc3)c4ccc(OC)c(c4)OC	56.0	7.3	970.0	6.0
22	GNF-Pf-4851		COc1ccc(cc1OC)C2CC(=O)C3=C(C2)NC(C)=C(C(=O)OC(C)C)C3c4cccc(Br)c4	> 5000	<5.40	> 5000	<5.40
23	GNF-Pf-5674		COc1ccc(cc1OC)C2CC(=O)C3=C(C2)NC(C)=C(C(=O)OC(C)C)C3c4ccc(Cl)cc4Cl	> 5000	<5.40	> 5000	<5.40
24	GNF-Pf-5501		COCCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3cccc(Cl)c3Cl)c4ccc(OC)c(c4)OC	1532.9	5.8	> 5000	<5.40
25	GNF-Pf-5446		COc1ccc(cc1OC)C2CC(=O)C4=C(C2)NC(C)=C(C(=O)OC3CCCC3)C4c5ccccc5OC	> 5000	<5.40	> 5000	<5.40
26	GNF-Pf-5436		COc1ccc(cc1OC)C2CC(=O)C4=C(C2)NC(C)=C(C(=O)OC3CCCC3)C4c5ccc(Cl)cc5	383.7	6.4	> 5000	<5.40
27	GNF-Pf-5621		COc1ccc(cc1OC)C2CC(=O)C3=C(C2)NC(C)=C(C(=O)OC(C)C)C3c4cccc(Cl)c4Cl	215.3	6.7	893.0	6.0
28	GNF-Pf-4529		COc1ccc(cc1OC)C2CC(=O)C3=C(C2)NC(C)=C(C(=O)OC(C)C)C3c4ccccc4F	540.1	6.3	> 5000	<5.40
29	GNF-Pf-5672		CCCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccc(Cl)cc3Cl)c4ccc(OC)c(c4)OC	185.6	6.7	> 5000	<5.40
30	GNF-Pf-5560		COCCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccc(F)cc3)c4ccc(OC)c(c4)OC	226.8	6.6	> 5000	<5.40
31	GNF-Pf-5572		COc1ccc(cc1OC)C2CC(=O)C3=C(C2)NC(C)=C(C(=O)OC(C)C)C3c4cccc(C)c4	857.2	6.1	> 5000	<5.40
32	GNF-Pf-5655		CCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccc(Cl)cc3Cl)c4ccc(OC)c(c4)OC	74.2	7.1	> 5000	<5.40
33	**GNF-Pf-5660**	**Vitas-M Laboratory, Ltd**	**CCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccccc3Cl)c4ccc(OC)c(c4)OC**	**14.4**	**7.8**	**132.0**	**6.8**
34	GNF-Pf-5670		CCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccc(Br)cc3)c4ccc(OC)c(c4)OC	645.2	6.2	> 5000	<5.40
35	GNF-Pf-4922, GNF-Pf-4026		COC(=O)c1ccc(cc1)C4C2C(=O)CC(CC=2Nc3ccccc3N4C(=O)C5=CC=CS5)c6ccc(C)cc6	> 5000	<5.40	> 5000	<5.40
36	GNF-Pf-2090		COc1ccc(cc1)c3cnnc(N/N=C/C2=CC=C(O2)[N+]([O-])=O)n3	1482.3	5.8	567.0	6.2
37	GNF-Pf-812		COc1ccc(cc1)C6Nc5ccc(Cc3ccc2NC(=Nc2c3)c4ccc(cc4)OC)cc5N=6	666.5	6.2	> 5000	<5.40
38	GNF-Pf-4252	Vitas-M Laboratory, Ltd	COc5cc4c(nc1c(C(=NN1c2ccccc2)c3ccccc3)c4cc5OC)c6cc(Br)ccc6O	120.5	6.9	489.0	6.3
39	GNF-Pf-5240	Vitas-M Laboratory, Ltd	CCOc1ccc(cc1)C4C3Nc2ccccc2C=3CCN4C(=O)C6=Cc5cc(Br)ccc5O6	137.8	6.9	899.0	6.0
40	GNF-Pf-4667		COC(=O)c1ccc(cc1)C4C2C(=O)CC(CC=2Nc3ccccc3N4C(=O)c5ccccc5)c6ccc(cc6)OC	> 5000	<5.40	> 5000	<5.40
41	GNF-Pf-1209		CC3C/C(=C\c1ccncc1)/C(=O)/C(=C/c2ccncc2)/C3	464.8	6.3	43.2	7.4
42	GNF-Pf-5460		Cc3cc(Nc1ccc(cc1)OCc2ccccc2)nc4cc(O)ccc34	> 5000	<5.40	> 5000	<5.40
43	nil, nil GNF-Pf-1823, GNF-Pf-3715		CC1OC=CC=1C(=O)N/N=C/C(/Br)=C/c2ccccc2	> 5000	<5.40	> 5000	<5.40
44	GNF-Pf-4508		CC(C)CCNCC(O)CN3c1ccc(Cl)cc1c2cc(Cl)ccc23	561.2	6.3	509.0	6.3
45	GNF-Pf-5639		COc1cc(ccc1OC)C2CC(=O)C4=C(C2)NC(C)=C(C(=O)OC3CCCC3)C4c5ccc(cc5)[N+]([O-])=O	> 5000	<5.40	> 5000	<5.40
46	GNF-Pf-4593		COc1ccc(cc1)Nc4nc(N/N=C/c2cccc(c2)[N+]([O-])=O)nc(Nc3ccc(cc3)[N+]([O-])=O)n4	852.9	6.1	> 5000	<5.40
47	GNF-Pf-4238		CN1CCN(CC1)c4nc(N/N=C/c2cc(I)ccc2O)nc(Nc3ccc(cc3)[N+]([O-])=O)n4	63.7	7.2	221.0	6.7
48	GNF-Pf-4194		CC1=CC=C(C)N1c2cc(ccc2)C(=O)N/N=C/C4C=C(c3ccccc3)N(C=4c5ccccc5)c6ccc(C)cc6	1733.2	5.8	2189.0	5.7
49	GNF-Pf-71		O=C(NCCC1CCCCC=1)c6cc5N=C(SCC3Nc2ccccc2N=3)N(c4ccccc4)C(=O)c5cc6	> 5000	<5.40	> 5000	<5.40
50	GNF-Pf-5543		CCC(C)OC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccc(Cl)cc3)c4ccc(Cl)cc4	279.5	6.6	> 5000	<5.40

Supplementary Table 1 (cont.). Gametocytocidal activity of the resourced selected screening hits.

51	GNF-Pf-4596		CCC2(C)CC(CCNCc1ccccc1O)(CCO2)c3ccccc3OC	634.0	6.2	750.0	6.1
52	GNF-Pf-4070		COc1nncc(n1)c2cnnc(n2)SC	382.6	6.4	> 5000	<5.40
53	GNF-Pf-3921, TCMDC-124347		CC2Nc1ccc(C)cc1C(=O)C=2Br	2786.1	5.6	> 5000	<5.40
54	GNF-Pf-3254		CC(=O)N(c1ccc(F)c(F)c1)C3C(=O)c2ccccc2C(=O)C=3N4CCOCC4	> 5000	<5.40	> 5000	<5.40
55	GNF-Pf-5498		CCC(C)OC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccccc3F)c4ccc(Cl)cc4	123.0	6.9	597.0	6.2
56	GNF-Pf-2288		CC(=O)N(c1cccc(Cl)c1)C3C(=O)c2ccccc2C(=O)C=3N4CCOCC4	3489.1	5.5	> 5000	<5.40
57	GNF-Pf-739		COC(=O)c1cc(cc(c1)C(=O)OC)NC(=S)NC(=O)c2c(cccc2OC)OC	> 5000	<5.40	> 5000	<5.40
58	GNF-Pf-5037		O=C2c1ccccc1Nc3ccc(F)cc23	> 5000	<5.40	> 5000	<5.40
59	GNF-Pf-5557		COc1cccc(n1)c3ccc(O)c(CNCC2CCCCC2)c3	738.9	6.1	> 5000	<5.40
60	GNF-Pf-5631		CC(C)CNCc1cc(ccc1O)c2ccc(F)cc2	636.8	6.2	803.0	6.1
61	GNF-Pf-5082		Oc1ccc(cc1CNC3C2CC4CC(C2)CC3C4)c5cccnc5	1899.5	5.7	> 5000	<5.40
62	GNF-Pf-5045		Oc1ccc(cc1CN2CCC(CC2)c3ccccc3)c4ccccc4	2007.9	5.7	> 5000	<5.40
63	GNF-Pf-4870		Oc1ccc(cc1CNCC2CC3C=CC2C34CC4)c6ccc5OCOc5c6	630.0	6.2	897.0	6.0
64	GNF-Pf-5616		COc1cccc(n1)c5ccc(O)c(CNCC2CC3C=CC2C34CC4)c5	450.1	6.3	560.0	6.3
65	GNF-Pf-5087		COc1ccc(OC)c(c1)c5ccc(O)c(CNCC2CC3C=CC2C34CC4)c5	669.7	6.2	1072.0	6.0
66	GNF-Pf-5659		COc1ccc(OC)c(c1)c4ccc(O)c(CN2CCC(CC2)c3ccccc3)c4	2048.9	5.7	> 5000	<5.40
67	GNF-Pf-2168		[O-][N+](=O)c4ccc3nc(Nc1ccccc1)c(Nc2ccccc2)nc3c4	> 5000	<5.40	> 5000	<5.40
68	GNF-Pf-279		COC(=O)C1=C(OC)C(=O)OC12C=CC(=O)C=C2	959.9	6.0	181.0	6.7
69	unknown		CC(=O)NC1=NC(C)=C(S1)C3=CSC(Nc2ccc(Cl)cc2)=N3	2068.0	5.7	> 5000	<5.40
70	GNF-Pf-5294	Vitas-M Laboratory, Ltd	CCNc1nc(NC(C)CC)nc(n1)ON=C(C)C	224.8	6.6	1431.0	5.8
71	GNF-Pf-1532		CCN(CC)c2ccc1C=C(/C(/C)=N/NC(N)=S)C(=O)Oc1c2	515.2	6.3	365.0	6.4
72	GNF-Pf-5427	ChemDiv	Oc1ccc(cc1CN2CCCCCC2)c3ccccc3	256.1	6.6	673.0	6.2
73	GNF-Pf-2634	AKos GmbH	Oc2cc1ccccc1cc2C(=O)Nc3cccc(Cl)c3	1088.3	6.0	2701.0	5.6
74	unknown		N=C3N(CCN1CCCCC1)c2ccccc2N3CC(O)c4ccc(Cl)c(Cl)c4	1131.7	5.9	1877.0	5.7
75	GNF-Pf-4917		OC(COc1ccccc1C(=O)Nc2ccccc2)CN5c3ccccc3c4ccccc45	1607.9	5.8	> 5000	<5.40
76	GNF-Pf-2943		[O-][N+](=O)C4=CC=C(/C=N/Nc3nc(Nc1ccccc1)nc(Nc2ccccc2)n3)O4	1186.0	5.9	549.0	6.3
77	GNF-Pf-3626		COc4ccc3c(C)nc(Nc1nc(C)cc(n1)c2ccccc2)nc3c4	> 5000	<5.40	> 5000	<5.40
78	GNF-Pf-4526		CN(C)CCCN4C(N)=C(C2Nc1ccccc1N=2)c3nc(C#N)c(C#N)nc34	961.8	6.0	1530.0	5.8
79	GNF-Pf-2567		CCN(CC)c2ccc(CN1CCN(CC)CC1)cc2	> 5000	<5.40	> 5000	<5.40
80	GNF-Pf-5666		CN1C(NNC(N)=S)C(NNC(N)=S)N(C)C1=O	21.3	7.7	1.3	8.9
81	GNF-Pf-2329		Cc2c1ccccc1c(C)c3ccccc23	2348.2	5.6	984.0	6.0
82	GNF-Pf-3600		CC(=O)N(c1ccc(F)cc1)C3C(=O)c2ccccc2C(=O)C=3N(C)C	243.3	6.6	588.0	6.2
83	GNF-Pf-4055		CC(=O)N(c1cccc(F)c1)C3C(=O)c2ccccc2C(=O)C=3N(C)C	3124.4	5.5	> 5000	<5.40
84	GNF-Pf-3020		CC(=O)N(c1cccc(Cl)c1)C3C(=O)c2ccccc2C(=O)C=3N(C)C	371.9	6.4	677.0	6.2
85	GNF-Pf-3202		CC(=O)N(c1ccc(F)c(F)c1)C3C(=O)c2ccccc2C(=O)C=3N(C)C	425.1	6.4	380.0	6.4
86	GNF-Pf-5634		C[C@@H]1CC[C@H]2[C@@H](C)[C@@H](O)O[C@@H]3O[C@]4(C)CC[C@@H]1C23OO4	3.4	8.5	8.8	8.1
87	GNF-Pf-4628, TCMDC-125542		Fc3cccc4SC(NC1=NC(=CS1)c2ccccn2)=Nc34	3818.2	5.4	625.0	6.2
88	GNF-Pf-3527, TCMDC-125541		COc4ccc3N=C(NC1=NC(=CS1)c2ccccn2)Sc3c4	4357.3	<5.40	262.0	6.6
89	GNF-Pf-4604		O=C(Nc1ccc(cc1)C3Nc2ccccc2N=3)c4cccc(n4)C(=O)Nc5ccc(cc5)C7Nc6ccccc6N=7	322.4	6.5	305.0	6.5
90	GNF-Pf-4378		CCN4/C(=N/C(=O)C2=CC(=O)c1ccccc1O2)/Sc3c(ccc(OC)c34)OC	> 5000	<5.40	> 5000	<5.40
91	GNF-Pf-4808		CC(C)(C)NCC(O)CN3c1ccc(Cl)cc1c2cc(Cl)ccc23	722.6	6.1	713.0	6.1
92	GNF-Pf-5458		CC2(C)C1CC(CC1)C2(C)NC(=S)N/N=C/c3ccccn3	> 5000	<5.40	9.2	8.0
93	GNF-Pf-5568	ChemDiv	Oc1ccc(cc1CNC2CCCCC2)c3ccc(F)cc3	212.5	6.7	538.2	6.3
94	GNF-Pf-5599		COc1ccc(OC)c(c1)c4ccc(O)c(CNCC2(CCCCC2)N3CCCCC3)c4	907.9	6.0	928.0	6.0
95	GNF-Pf-4755		O=C(NC1CC1)C2CCN(CC2)c4cccc5C(=O)N(C3CCCCCCC3)C(=O)c45	> 5000	<5.40	> 5000	<5.40
96	GNF-Pf-5546		CC(C)OC(=O)c1c(nc(nc1C)c2ccccc2)C(=O)N4CCN(C(=O)NC3CCCCC3)C(C)C4	> 5000	<5.40	> 5000	<5.40
97	GNF-Pf-5077		CC2C=CN1C(NC(C)(C)CC(C)(C)C)=C(N=C1C=2)c3ccccc3OC(=O)c4ccc(Br)cc4	> 5000	<5.40	> 5000	<5.40
98	GNF-Pf-5072		COc1cc(cc(OC)c1OC)Nc2cncc(n2)c3ccc(cc3)C(N)=O	270.4	6.6	639.0	6.2
99	GNF-Pf-5356		N#Cc1ccc(cc1)c2cnc(N)c(n2)c3ccc(cc3)C(N)=O	> 5000	<5.40	> 5000	<5.40
100	GNF-Pf-188		N=C2NC(=O)/C(=C/C=C/C1=CC=C(O1)[N+]([O-])=O)/S2	> 5000	<5.40	820.0	6.1

Supplementary Table 1 (cont.). Gametocytocidal activity of the resourced selected screening hits.

101	GNF-Pf-4325		COc2ccc(CC1NCC(O)C1OC(C)=O)cc2	46.8	7.3	568.0	6.2
102	MMV645672, TCMDC-123496, GNF-Pf-4583		OC4c1ccccc1C5=Nc2ccccc2SC(c3ccccc3)C=45	> 5000	<5.40	> 5000	<5.40
103	GNF-Pf-4816	AKos GmbH	CCCCCCc3cc(O)c2C1CC(C)CCC=1C(=O)Oc2c3	1667.1	5.8	5133.0	5.3
104	GNF-Pf-5523		CCCN(CCC)CC(O)COc1ccccc1C(=O)Nc2ccccc2	685.2	6.2	1400.0	5.9
105	GNF-Pf-3683		OC(CNC1CCCCC1)CN4c2ccccc2c3ccccc34	1403.3	5.9	> 5000	<5.40
106	GNF-Pf-4323		O=C(NC1=NC(=CS1)c2ccccn2)c3ccc(cc3)Oc4ccc(cc4)C(=O)NC5=NC(=CS5)c6ccccn6	535.0	6.3	99.0	7.0
107	GNF-Pf-5629	AKos GmbH	CCOC(=O)C2=C(NC(=O)c1ccccc1)Sc3c(O)c(CN(CC)CC)ccc23	224.3	6.6	438.0	6.4
108	GNF-Pf-3195	Vitas-M Laboratory, Ltd	COc2cc1nc(nc(N)c1cc2OC)N3CCN(CC3)c5nc(N)c4cc(OC)c(cc4n5)OC	154.7	6.8	330.0	6.5
109	GNF-Pf-3625		CN5C=C(C(C1=CC=CS1)C2=CN(C)c3ccccc23)c4ccccc45	> 5000	<5.40	2055.0	5.7
110	GNF-Pf-4773		C2SC(Nc1ccccn1)=NC=2c3ccccn3	> 5000	<5.40	51.0	7.3
111	GNF-Pf-3984		COc5ccc(CNCC3=CC2C(=NN(c1ccccc1)C=2NC3=O)c4ccc(C)cc4)cc5	> 5000	<5.40	> 5000	<5.40
112	unknown		Cc1ccc(C)c(c1)Nc4nc(NCc2ccccc2)c3ccccc3n4	2135.0	5.7	> 5000	<5.40
113	unknown		CC(C)(C)C3=NN(c1ccc(Cl)cc1)C4NC(=O)C(CNCc2ccccc2)=CC3=4	1219.2	5.9	1242.0	5.9
114	unknown		CC(C)(C)C3=NN(c1ccc(Cl)cc1)C4NC(=O)C(CNCc2ccc(Cl)cc2)=CC3=4	223.5	6.7	534.0	6.3
115	GNF-Pf-3366	AKos GmbH	Cc4ccc(NC(=O)C1=CC(=CN(C1=O)c2ccc(C)cc2C)C(=O)c3cc(Cl)ccc3O)c(C)c4	163.1	6.8	534.0	6.3
116	GNF-Pf-1621	AKos GmbH	Cc1ccc(cc1C)NC(=O)C2=CC(=CN(C2=O)c3ccc(C)c(C)c3)C(=O)c4cc(Cl)ccc4O	150.5	6.8	278.0	6.6
117	GNF-Pf-5323	Ryan Scientific Inc.	COc1ccccc1N3CCN(Cc2cc(C(C)C)c(C)cc2O)CC3	423.8	6.4	1597.0	5.8
118	GNF-Pf-5527, SJ000087525		CCCN(C(=O)C(=O)Nc3ccc2N=C1CCCCCN1C(=O)c2c3)c4ccc(cc4)OCC	500.4	6.3	> 5000	<5.40
119	GNF-Pf-5661		CCOC(=O)C3C=C(c1ccc(cc1)OC)N(CCC(=O)Nc2cccc(c2)C(C)=O)C=3C	1814.0	5.7	> 5000	<5.40
120	GNF-Pf-5392	AKos GmbH	CCOC(=O)C3C=C(c1ccc(F)cc1)N(CCC(=O)Nc2cccc(c2)C(=O)OCC)C=3C	789.7	6.1	1763.0	5.8
121	GNF-Pf-5115	AKos GmbH	CCN(C(=O)CSC1=NC(=NO1)c2ccc(cc2)C(C)C)c3cccc(C)c3	502.7	6.3	1758.0	5.8
122	GNF-Pf-3531		COc1ccc(cc1)C3N=C(NC(=O)CS(=O)(=O)c2ccc(F)cc2)SC=3C	> 5000	<5.40	> 5000	<5.40
123	GNF-Pf-4133		CN(C)CCNc1cc(nc2ccccc12)c4ccc3ccccc3c4	> 5000	<5.40	> 5000	<5.40
124	GNF-Pf-4406, GNF-Pf-3381		CCN5/C(=C/c2ccc1ccccc1[n+]2CC)/C=CC4Sc3ccccc3C=45	> 5000	<5.40	123.0	6.9
125	GNF-Pf-5483		Cc2cc(N)c1ccccc1[n+]2CCCCCCCCCC[n+]4c3ccccc3c(N)cc4C	1036.7	6.0	> 5000	<5.40
126	GNF-Pf-2812		COc1ccc(cc1)c4cc3C(=O)c2ccccc2c3nn4	373.4	6.4	> 5000	<5.40
127	GNF-Pf-4739, TCMDC-125769		Cc1ccnc(c1)NC2=NC(=CS2)c3ccccn3	584.9	6.2	68.0	7.2
128	**GNF-Pf-5668**	**Vitas-M Laboratory, Ltd**	**CCCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccccc3F)c4ccc(OC)c(c4)OC**	**20.1**	**7.7**	**125.6**	**6.8**
129	GNF-Pf-1950		CC3OC2C(=O)c1ccccc1C(=O)C=2C=3C(=O)NC4CCS(=O)(=O)C4	> 5000	<5.40	1380.0	5.9
130	MMV665980, GNF-Pf-4228		OC1CCCCC1C2(CCCCC2)NC(=S)Nc3ccccc3	> 5000	<5.40	> 5000	<5.40
131	GNF-Pf-3427		CN1CCN(CC1)c2ccc(cc2)Nc5c3ccccc3nc4ccccc45	1203.6	5.9	> 5000	<5.40
132	MMV007907, GNF-Pf-3677		Cc1ccc(cc1)NC2=NC(=CS2)c3ccccn3	> 5000	<5.40	352.0	6.5
133	GNF-Pf-5633		CCCOC(=O)C2=C(C)NC1CC(CC(=O)C=1C2c3ccc(Cl)cc3Cl)c4ccc(cc4)OC	509.4	6.3	> 5000	<5.40
134	GNF-Pf-5533		CCCCCC(=O)N4C(c1ccc(cc1)C(F)(F)F)C2C(=O)CC(CC=2Nc3ccccc34)c5ccc(OC)c(c5)OC	285.6	6.5	> 5000	<5.40
135	GNF-Pf-4736, TCMDC-123596		COc1ccc(cc1)C4C(C(=O)Nc2cc(Cl)c(cc2OC)OC)c3ccccc3C(=O)N4C	2922.0	5.5	> 5000	<5.40
136	GNF-Pf-4229		Cc3ccc(/N=N/C2=Nc1ccccc1N2C)c(C)c3	264.4	6.6	> 5000	<5.40
137	GNF-Pf-4543, MMV006389		O=C4c1ccccc1C5=Nc2ccccc2SC(c3cccc(F)c3)C45	> 5000	<5.40	> 5000	<5.40
138	GNF-Pf-5637	ChemDiv	C=CC1CN2CCC1C(C2)C(O)c3ccnc4ccc(cc34)OC	114.5	6.9	366.0	6.4
139	**GNF-Pf-5310**	**Ryan Scientific Inc.**	**CC(C)(C)c2ccc(O)c(CNC1CCCCC1)c2**	**54.1**	**7.3**	**163.6**	**6.7**
140	GNF-Pf-5561		C=CC12CCN(CC1)C(C2)C(O)c3ccnc4ccc(cc34)OC	199.6	6.7	238.0	6.6
141	unknown		CC(C)N2CCC1C(C(N)=O)=C(NC(=O)C(C)(C)C)SC=1C2	> 5000	<5.40	> 5000	<5.40
142	GNF-Pf-3788		CNC2C(=O)c1ccccc1C(=O)C=2N(Cc3ccc(Cl)c(Cl)c3)C(C)=O	254.4	6.6	30.0	7.5
143	same as NVP-LJA590-AA-1		C=CC1CN2CCC1CC2[C@H](O)c3ccnc4ccc(cc34)OC	526.9	6.3	> 5000	<5.40
144	GNF-Pf-237		O=C(c1ccncc1)N2N=C(CC2c3ccccc3)C5C(=O)Nc4ccc(Br)cc4C=5c6ccccc6	> 5000	<5.40	> 5000	<5.40
145	MMV007127, GNF-Pf-5290		OC4c1ccccc1C5=Nc2ccccc2SC(c3ccc(Br)cc3)C=45	> 5000	<5.40	> 5000	<5.40
146	GNF-Pf-4684, TCMDC-125391, GNF-Pf-4105		CN(C)c2ccc(/C=C/C(=O)NC(=O)c1ccccc1O)cc2	> 5000	<5.40	70.7	7.2
147	GNF-Pf-2632, GNF-Pf-4835		CCN(CC)c2ccc(/C=C/c1cccc[n+]1CC)cc2	> 5000	<5.40	366.0	6.4
148	TCMDC-124060, GNF-Pf-5669		OC4c1ccccc1C5=Nc2ccccc2SC(C3=CC=CS3)C=45	> 5000	<5.40	1540.0	5.8
149	GNF-Pf-5371		COc1ccc(cc1)C(OCCS(=O)(=O)CCO)(c2ccccc2)c3ccc(cc3)OC	> 5000	<5.40	> 5000	<5.40

The compounds selected for profiling are highlighted. Commercial source is indicated for compounds that were purchased as solids, remaining compounds were provided by NITD as DMSO stocks.Early GAM: stage I gametocyte; late GAM: stage IV gametocyte; pIC50: equals to -log(IC50). Early GAM: stage I gametocyte; late GAM: stage IV gametocyte; pIC50: equals to -log(IC50).

Supplementary Table 2. Activity of early- and late-stage GAM assay controls.

	NF54^{Pfs16} early GAM		NF54^{Pfs16} late GAM	
	mean IC_{50} (µM)	SEM	mean IC_{50} (µM)	SEM
Puromycin	0.096	0.012	0.043	0.004
Dihydroartemisinin	0.006	0.001	ND	-
Chloroquine	0.164	0.043	> 40 µM	-
Pyrimethamine	> 40 µM	-	> 40 µM	-
Primaquine	> 20 µM	-	ND	-

GAM: gametocytes; ND: not done; SEM: standard error of the mean based on 2 independent repeats performed in triplicate or duplicate for each parasite stage.

Supplementary Table 3. Activity profile of the 4 compounds of interest identified by screening the GNF-Novartis Malaria Box.

	Gametocyte IC_{50} (nM)				Asexual blood stage IC_{50} (nM)						
	NF54[Pfs16] early GAM		NF54[Pfs16] late GAM		3D7		Dd2		NITD609-R[Dd2] #2		HEK-293
	mean IC_{50}	SEM	mean IC_{50}	SEM	mean IC_{50}	SEM	mean IC_{50}	SEM	mean IC_{50}	SEM	cytotoxicty
GNF-Pf-5640	15.1	0.9	92.1	14.4	4.0	0.4	7.5	1.3	7.8	1.1	50%
GNF-Pf-5660	14.4	0.3	132.0	50.5	3.3	0.4	3.6	1.1	5.4	0.8	<5%
GNF-Pf-5668	20.1	0.5	125.6	40.4	5.5	0.5	6.9	1.2	11.2	1.8	45%
GNF-Pf-5310	54.1	2.2	163.6	40.4	22.1	1.9	24.0	6.3	21.7	2.9	41%

Early and late GAM stages refer to stages I and IV respectively. SEM: standard error of the mean based on 2 independent repeats performed in triplicate or duplicate for each parasite stage and strain. HEK-293 values show the percent inhibition at 40 μM.

Supplementary Table 4a. Transmission-blocking activity of HHQs.

			% reduction infected mosquitoes				% reduction oocyst numbers				% inhibition exflagellation			
assay	drug	presssure	mean IC_{50}	SEM	n	*P* value	mean IC_{50}	SEM	n	*P* value	mean IC_{50}	SEM	n	*P* value
in vitro	GNF-Pf-5660	1x IC_{50}	-18.5	12.9	2	>0.9999	58.1	15.4	2	0.0985	22.1	26.7	2	>0.9999
		3x IC_{50}	97.2	2.8	2	<0.0001	100.0	0.1	2	0.0019	97.7	2.3	2	0.0104
	GNF-Pf-5668	1x IC_{50}	18.0	12.4	2	>0.9999	67.1	4.0	2	0.0416	47.5	33.9	2	0.5127
		3x IC_{50}	57.1	11.2	2	0.0027	95.0	3.7	2	0.0030	70.2	8.9	2	0.0903
	GNF-Pf-5310	1x IC_{50}	-5.3	23.4	2	>0.9999	14.1	52.2	2	>0.9999	19.4	14.7	2	>0.9999
		3x IC_{50}	7.2	7.8	2	>0.9999	73.4	4.4	2	0.0228	-8.7	41.3	2	>0.9999
	MB	3x IC_{50}	100.0	0.0	2	<0.0001	100.0	0.0	2	0.0019	100.0	-	1	0.0366
in vivo	GNF-Pf-5660	100 mg/kg	24.7	21.0	2	0.5676	21.1	44.2	2	>0.9999	-0.1	16.9	2	>0.9999
	GNF-Pf-5668	100 mg/kg	1.6	3.0	2	>0.9999	6.8	33.3	2	>0.9999	31.1	1.2	2	0.0585
	DHA	20 mg/kg	64.8	19.7	2	0.0245	75.1	22.4	2	0.2174	-11.4	2.1	2	0.8744

In vitro assays were performed using the standard membrane feeding assay with *P. falciparum* parasites and the *in vivo* assay involved CD1 mice infected with *P. berghe*i parasites. Statistical significance was assessed using a Welch's corrected t-test with Bonferroni-Dunn adjusted *P* values for multiple comparisons when applicable. MB: methylene blue; DHA: dihydroartemisinin; SEM: standard error of the mean.

Supplementary Table 4b. *In vivo* asexual blood stage activity of HHQs.

			4-day Peters test				2-dose test			
			mean IC_{50}	SEM	n	*P* value	mean IC_{50}	SEM	n	*P* value
in vivo	GNF-Pf-5660	70 mg/kg	21.5	1.2	2	< 0.0001	-	-	-	-
		100 mg/kg	65.0	-	1	< 0.0001	7.7	1.7	2	>0.9999
	GNF-Pf-5668	70 mg/kg	42.8	1.1	2	< 0.0001	-	-	-	-
		100 mg/kg	91.1	-	1	< 0.0001	34.9	16.5	2	0.0323
	DHA	30 mg/kg	98.7	1.0	3	< 0.0001	99.6	0.4	2	0.0001

Data are expressed as % inhibition versus a DMSO-treated control. Statistical significance was assessed using a Welch's corrected t-test with Bonferroni-Dunn adjusted *P* values for multiple comparisons. ABS: asexual blood stage; DHA: dihydroartemisinin; SEM: standard error of the mean.

Supplementary Table 4c. Detailed *in vivo* transmission-blocking data of all treatment groups.

experiment	mouse	% infected mosquitoes				average # oocysts / infected mosquito				average exflagellation centers per 100 cells			
		solvent	GNF-Pf-5660	GNF-Pf-5668	DHA	solvent	GNF-Pf-5660	GNF-Pf-5668	DHA	solvent	GNF-Pf-5660	GNF-Pf-5668	DHA
1	1	75.0	83.3	80.0	20.0	62.9	34.6	26.1	1.8	5.8	3.8	1.3	5.1
	2	95.0	85.0	95.0	25.0	56.2	82.2	70.3	1.6	4.1	5.4	2.2	5.1
	3	100.0	90.0	90.0	10.0	29.4	36.4	39.9	1.0	4.3	2.6	3.4	4.7
	4	85.0	83.3	95.0	0.0	25.0	60.3	82.9	0.0	3.4	2.8	5.4	-
2	1	30.0	23.1	28.6	27.3	3.5	1.0	1.5	1.0	7.2	7.1	5.4	4.0
	2	50.0	35.0	63.6	20.0	8.5	2.4	3.0	1.7	4.0	10.8	2.4	6.9
	3	52.9	20.0	50.0	36.4	3.2	1.3	3.3	4.3	7.0	4.3	7.2	6.8
	4	65.0	29.4	46.7	25.0	2.5	1.4	2.9	1.5	7.1	-	2.1	9.9

Supplementary Table 5. IC_{50} data of the selected lines and genetically engineered (edited) lines against HHQs.

	GNF-Pf-5640					GNF-Pf-5660					GNF-Pf-5668				
Line (allele balance)	Mean IC_{50}	SEM	n	Fold shift vs wild-type	*P* value	Mean IC_{50}	SEM	n	Fold shift vs wild-type	*P* value	Mean IC_{50}	SEM	n	Fold shift vs wild-type	*P* value
Dd2-B2	13.2	2.1	10	1.0	n/a	15.1	1.6	8	1.0	n/a	23.0	3.9	8	1.0	n/a
5668-3C4D2 (F1072L 69%)*	442.7	43.8	4	33.7	8.268E-09	939.8	115.5	5	62.1	2.949E-06	846.1	85.2	5	36.8	4.459E-07
F1072L edited (F1072L 100%)	588.2	37.2	7	44.7	5.165E-11	892.2	18.0	3	58.9	1.27E-13	908.1	26.1	3	39.5	5.875E-12
5640-2C4C3 (S1075I 59%)	1012.4	107.3	4	77.0	1.36E-08	658.8	81.1	5	43.5	3.219E-06	1623.8	107.3	5	70.7	4.501E-09
S1075I edited (S1075I 100%)	1460.8	49.6	3	111.1	2.4E-14	642.0	22.1	3	42.4	1.514E-11	1742.1	113.4	3	75.8	3.487E-09
5640-3H9C7 (Y290F 50%)	1039.1	171.2	4	79.0	1.886E-06	2792.2	148.7	5	184.4	3.952E-10	1955.2	183.6	5	85.1	1.791E-07
5660-2B6H1 (Y290F 100%)	1382.6	163.0	4	105.1	4.393E-08	3750.2	338.7	5	247.7	1.094E-07	2373.4	89.5	5	103.3	9.979E-12
NF54	6.7	0.2	4	1.0	n/a	12.0	0.4	4	1.0	n/a	9.9	0.5	4	1.0	n/a
NF54-Y290F (Y290F 100%)	2239.3	29.9	3	332.4	0.0002	5247.5	163.2	4	437.2	<0.0001	2313.7	19.0	3	233.1	<0.0001
FCB	9.8	1.0	4	1.0	n/a	18.3	1.5	4	1.0	n/a	12.4	1.1	4	1.0	n/a
$KD1^{mdr1}$ (*pfmdr1* knock-down)	9.7	1.1	4	1.0	0.9368	17.1	1.3	4	0.9	0.6	12.4	0.4	4	1.0	0.9886

All values are given in nM. *: This strain also has four copies of *pfmdr1,*compared to two copies in the Dd2-B2 parent; SEM: standard error of the mean; n: number of repeated experiments; *P* value vs Dd2-B2: Welch's t-test corrected for multiple comparisons using the Bonferroni-Dunn method; *P* value vs NF54 or FCB: Welch's t test; n/a.: not applicable.

Supplementary Table 6. IC_{90} data of the selected lines and genetically engineered (edited) lines against HHQs.

	GNF-Pf-5640					GNF-Pf-5660					GNF-Pf-5668				
Line (allele balance)	Mean IC_{90}	SEM	n	Fold shift vs wild-type	*P* value	Mean IC_{90}	SEM	n	Fold shift vs wild-type	*P* value	Mean IC_{90}	SEM	n	Fold shift vs wild-type	*P* value
Dd2-B2	47.0	4.1	12	1.0	n/a	67.1	4.7	8	1.0	n/a	97.4	30.2	8	1.0	n/a
5668-3C4D2 (F1072L 69%)*	2553.5	157.9	4	54.3	3.04E-13	3792.2	452.4	5	56.5	2.227E-06	2766.8	172.0	5	28.4	4.511E-09
F1072L edited (F1072L 100%)	2675.3	239.2	7	56.9	2.587E-10	3486.0	338.9	3	52.0	1.229E-07	2645.0	95.4	3	27.2	4.033E-10
5640-2C4C3 (S1075I 59%)	7317.0	2600.1	4	155.5	0.0007633	2402.6	290.7	5	35.8	2.858E-06	4654.8	270.6	5	47.8	1.448E-09
S1075I edited (S1075I 100%)	3648.7	87.6	3	77.6	<01E-15	1950.3	104.4	3	29.1	7.678E-10	3376.7	174.5	3	34.7	1.468E-09
5640-3H9C7 (Y290F 50%)	6451.8	1785.1	4	137.1	5.945E-05	11018.6	3792.1	5	164.2	0.0190308	4217.8	308.0	5	43.3	1.631E-08
5660-2B6H1 (Y290F 100%)	6241.3	901.3	4	132.7	2.307E-08	10029.2	1475.6	5	149.5	1.596E-05	4676.4	92.1	5	48.0	3.7E-14
NF54	15.8	0.7	4	1.0	n/a	31.0	1.9	4	1.0	n/a	24.4	1.2	4	1.0	n/a
NF54-Y290F (Y290F 100%)	4123.3	259.0	3	260.9	0.0040	11209.7	208.0	3	361.4	0.0003	4350.33	316.2	3	178.1	0.0053
FCB	50.3	4.8	4	1.0	n/a	152.4	38.6	4	1.0	n/a	93.2	26.2	4	1.0	n/a
$KD1^{mdr1}$ (*pfmdr1* knock-down)	33.9	0.8	4	0.7	0.0393	84.8	3.0	4	0.6	0.1780	57.4	6.6	4	0.6	0.2681

All values are given in nM. *: This strain also has four copies of *pfmdr1,* compared to two copies in the Dd2-B2 parent; SEM: standard error of the mean; n: number of repeated experiments; *P* value vs Dd2-B2: Welch's t-test corrected for multiple comparisons using the Bonferroni-Dunn method; *P* value vs NF54 or FCB: Welch's t test; n/a.: not applicable.

Supplementary Table 7. IC_{50} data of early GAM stage NF54 and NF54-Y290F lines against HHQs.

Line (allele balance)	GNF-Pf-5640 Mean IC_{50}	SEM	n	Fold shift vs wild-type	*P* value	GNF-Pf-5660 Mean IC_{50}	SEM	n	Fold shift vs wild-type	*P* value	GNF-Pf-5668 Mean IC_{50}	SEM	n	Fold shift vs wild-type	*P* value
NF54	192.1	133.9	2	1.0	n/a	249.8	122.3	2	1.0	n/a	293.3	98.4	2	1.0	n/a
NF54-Y290F (Y290F 100%)	14251.5	2492.5	2	74.2	0.0301	10063.5	921.5	2	40.3	0.0089	13943.5	973.5	2	47.5	0.0051

All values are given in nM. *: SEM: standard error of the mean; n: number of repeated experiments; *P* value vs NF54: unpaired t-test.

Supplementary Table 8. Minimum estimated energy required for docking of HHQs to wild-type and mutated PfMDR1.

Compound	PfMDR1	Minimum estimated energy (kcal/mol)
GNF-Pf-5640	wild-type	-5.44
	290F	-6.08
	1072L	-5.25
	1075I	-6.20
GNF-Pf-5660	wild-type	-6.46
	290F	-6.49
	1072L	-7.30
	1075I	-6.23
GNF-Pf-5668	wild-type	-5.96
	290F	-7.00
	1072L	-7.71
	1075I	-5.90

Supplementary Table 9. Interactions of GNF-Pf-5640 with wild-type and mutated PfMDR1.

wild-type PfMDR1

hydrogen bonds	hydrophobic	pi-pi	other
N1 (*6*) [*3.29*] - TYR803 (*OH*)	C27 (*33*) [*3.54*] - ILE286 (*CG2*)	C19 (*25*) [*3.35*] - TYR810 (*CD2, CE2*)	O3 (*3*) [*3.48*] - ILE286 (*CG2*)
H1 (*36*) [*2.96*] - TYR803 (*OH*)	C26 (*32*) [*3.51*] - LEU287 (*CD2*)	C24 (*30*) [*3.58*] - TYR810 (*CD2*)	C10 (*16*) [*3.87*] - TYR803 (*OH*)
	C27 (*33*) [*3.51*] - LEU287 (*CD2*)	C19 (*25*) [*3.45*] - PHE1068 (*CD2, CE2*)	O4 (*4*) [*3.50*] - ILE1071 (*CG2*)
	C27 (*33*) [*3.24*] - TYR290 (*CB, CG*)	C24 (*30*) [*3.16*] - PHE1068 (*CD2, CE2*)	C28 (*34*) [*2.97*] - SER1075 (*CB, OG*)
	C26 (*32*) [*3.17*] - PHE806 (*CD2, CE2*)	C22 (*28*) [*3.41*] - PHE1068 (*CD2, CE2*)	
	C27 (*33*) [*3.68*] - PHE806 (*CE2*)	C13 (*19*) [*3.53*] - PHE1072 (*CE1*)	
	C26 (*32*) [*3.59*] - TYR810 (*CB, CD1, CG*)		
	C28 (*34*) [*3.40*] - ILE1071 (*CG2*)		
	C28 (*34*) [*3.63*] - PHE1072 (*CD1*)		
	C1 (*7*) [*3.60*] - PHE1072 (*CE1*)		
	C2 (*8*) [*3.64*] - PHE1072 (*CE1, CZ*)		

PfMDR1-290F

polar	hydrophobic	pi-pi	other
O4 (*4*) [*3.84*] - SER1075 (*OG*)	C16 (*22*) [*3.39*] - ILE286 (*CD1, CG2*)	C22 (*28*) [*3.82*] - TYR803 (*CZ*)	O3 (*3*) [*3.66*] - PHE290 (*CD1, CE1, CZ*)
	C27 (*33*) [*3.61*] - ILE286 (*CG2*)	C17 (*23*) [*3.84*] - TYR803 (*CZ*)	C2 (*8*) [*3.84*] - TYR803 (*OH*)
	C27 (*33*) [*3.37*] - LEU287 (*CD2*)	C19 (*25*) [*3.75*] - PHE1068 (*CD2*)	C3 (*9*) [*3.86*] - TYR803 (*OH*)
	C27 (*33*) [*3.12*] - PHE290 (*CB, CD2, CG*)	C13 (*19*) [*3.69*] - PHE1072 (*CE1*)	C5 (*11*) [*3.63*] - TYR803 (*OH*)
	C25 (*31*) [*3.81*] - PHE290 (*CG*)		C22 (*28*) [*3.59*] - TYR803 (*OH*)
	C27 (*33*) [*3.56*] - PHE806 (*CE2*)		C17 (*23*) [*3.15*] - TYR803 (*OH*)
	C24 (*30*) [*3.21*] - ALA807 (*CB*)		O1 (*1*) [*3.44*] - PHE1072 (*CE1, CZ*)
	C26 (*32*) [*3.19*] - TYR810 (*CD1, CD2, CE1, CG, CZ*)		O4 (*4*) [*3.75*] - SER1075 (*CB*)
	C21 (*27*) [*3.31*] - PHE1068 (*CD2, CE2, CZ*)		
	C1 (*7*) [*3.25*] - PHE1072 (*CE1, CZ*)		
	C6 (*12*) [*3.30*] - PHE1072 (*CE1, CZ*)		
	C7 (*13*) [*3.35*] - PHE1072 (*CE1, CZ*)		

PfMDR1-1072L

polar	hydrophobic	pi-pi	other
O1 (*1*) [*3.28*] - TYR803 (*OH*)	C26 (*32*) [*3.12*] - ILE286 (*CG2*)	C19 (*25*) [*3.75*] - TYR803 (*CE1, CZ*)	C3 (*9*) [*3.57*] - TYR803 (*OH*)
	C25 (*31*) [*3.88*] - LEU287 (*CD2*)	C24 (*30*) [*3.22*] - TYR803 (*CE1, CZ*)	C6 (*12*) [*3.78*] - TYR803 (*OH*)
	C26 (*32*) [*3.63*] - LEU287 (*CD2*)	C22 (*28*) [*3.41*] - TYR803 (*CE1, CZ*)	C7 (*13*) [*3.19*] - TYR803 (*OH*)
	C27 (*33*) [*3.78*] - LEU287 (*CD2*)	C19 (*25*) [*2.88*] - PHE806 (*CD2, CE2*)	C11 (*17*) [*3.44*] - TYR803 (*OH*)
	C26 (*32*) [*3.35*] - TYR290 (*CB, CG*)	C24 (*30*) [*3.27*] - PHE806 (*CD2, CE2*)	C12 (*18*) [*3.83*] - TYR803 (*OH*)
	C25 (*31*) [*3.77*] - PHE806 (*CE2*)		C24 (*30*) [*3.88*] - TYR803 (*OH*)
	C26 (*32*) [*3.81*] - PHE806 (*CE2*)		C22 (*28*) [*3.50*] - TYR803 (*OH*)
	C27 (*33*) [*3.53*] - PHE806 (*CE2*)		C17 (*23*) [*3.24*] - TYR803 (*OH*)
	C21 (*27*) [*3.22*] - ALA807 (*CB*)		O3 (*3*) [*3.84*] - TYR810 (*CE2*)
	C27 (*33*) [*3.34*] - TYR810 (*CD1, CE1, CG*)		O1 (*1*) [*3.45*] - LEU1072 (*CD1, CD2*)
	C28 (*34*) [*3.46*] - ILE1071 (*CG2*)		C29 (*35*) [*3.65*] - SER1075 (*CB*)
	C6 (*12*) [*3.11*] - LEU1072 (*CD2, CG*)		
	C1 (*7*) [*3.75*] - LEU1072 (*CD2*)		
	C7 (*13*) [*3.88*] - LEU1072 (*CD2*)		
	C15 (*21*) [*3.78*] - LEU1072 (*CD2*)		

PfMDR1-1075I

hydrophobic	pi-pi	other
C26 (*32*) [*3.27*] - ILE286 (*CG2*)	C22 (*28*) [*3.64*] - TYR803 (*CZ*)	O2 (*2*) [*3.82*] - ILE286 (*CG2*)
C25 (*31*) [*3.90*] - LEU287 (*CD2*)	C24 (*30*) [*3.64*] - PHE806 (*CD2*)	O3 (*3*) [*3.37*] - TYR290 (*CD2, CE2, CZ*)
C26 (*32*) [*3.64*] - LEU287 (*CD2*)	C9 (*15*) [*3.80*] - PHE1072 (*CE1*)	C22 (*28*) [*3.43*] - TYR803 (*OH*)
C27 (*33*) [*3.27*] - LEU287 (*CD2*)		C17 (*23*) [*3.38*] - TYR803 (*OH*)
C27 (*33*) [*3.33*] - TYR290 (*CB, CD1, CG*)		O1 (*1*) [*3.24*] - PHE1068 (*CD1, CD2, CE1, CG, CZ*)
C25 (*31*) [*3.81*] - PHE806 (*CE2*)		O1 (*1*) [*3.43*] - PHE1072 (*CZ*)
C26 (*32*) [*3.56*] - PHE806 (*CE2*)		
C27 (*33*) [*3.41*] - PHE806 (*CE2, CZ*)		
C19 (*25*) [*3.60*] - ALA807 (*CB*)		
C24 (*30*) [*3.31*] - ALA807 (*CB*)		
C27 (*33*) [*3.87*] - TYR810 (*CD1*)		
C21 (*27*) [*3.35*] - TYR810 (*CD2, CE2*)		
C21 (*27*) [*3.49*] - PHE1068 (*CD2, CE2*)		
C1 (*7*) [*3.32*] - PHE1072 (*CE1, CZ*)		
C6 (*12*) [*3.42*] - PHE1072 (*CE1, CZ*)		
C7 (*13*) [*3.33*] - PHE1072 (*CZ*)		

Atom names and their corresponding number in brackets corresponds to the numbering of HHQ atoms in Supplementary Figure 3. Numbers in the square brackets indicate the lowest interatomic distance between the given ligand atom and the amino acid residue.

Supplementary Table 10. Interactions of GNF-Pf-5660 with wild-type and mutated PfMDR1.

wild-type PfMDR1

polar	hydrophobic	pi-pi	other
O2 (3) [3.60] - TYR803 (OH)	C14 (21) [3.39] - ILE286 (CD1)	C23 (30) [3.71] - TYR290 (CE2, CZ)	C26 (33) [3.70] - ASN283 (CB, OD1)
O5 (6) [3.60] - SER1075 (OG)	C24 (31) [3.68] - LEU287 (CD2, CG)	C22 (29) [3.81] - TYR290 (CE2)	O3 (4) [3.36] - ILE286 (CG2)
	C26 (33) [3.63] - LEU287 (CG)	C21 (28) [3.60] - TYR810 (CD2, CE2, CG)	O2 (3) [3.30] - TYR803 (CE1, CZ)
	C24 (31) [3.59] - TYR803 (CE1)	C23 (30) [3.46] - TYR810 (CE1, CE2, CZ)	C5 (12) [3.74] - TYR803 (OH)
	C24 (31) [3.42] - PHE806 (CD2, CE2)	C20 (27) [3.35] - PHE1072 (CD1, CE1)	C8 (15) [3.74] - TYR803 (OH)
	C27 (34) [3.44] - ILE1071 (CG2)	C19 (26) [3.37] - PHE1072 (CD1, CE1)	O3 (4) [3.80] - PHE806 (CE2)
	C7 (14) [3.85] - PHE1072 (CZ)	C18 (25) [3.62] - PHE1072 (CE1)	C23 (30) [3.82] - TYR810 (OH)
		C13 (20) [3.66] - PHE1072 (CE1)	O5 (6) [3.84] - ILE1071 (CG2)
			O5 (6) [3.62] - PHE1072 (CD1, CE1)
			O1 (2) [3.31] - PHE1072 (CZ)
			C27 (34) [3.55] - SER1075 (CB, OG)
			C25 (32) [3.80] - TYR1076 (OH)
			C25 (32) [3.86] - LYS1079 (NZ)

PfMDR1-290F

hydrogen bonds	polar	hydrophobic	pi-pi	halogen-bond	other
N1 (7) [2.90] - ASN283 (CG, OD1)	O1 (2) [3.43] - TYR803 (OH)	C17 (24) [3.90] - ILE286 (CD1)	C3 (10) [3.67] - TYR803 (CE1)	Cl1 (1) [3.72] - TYR803 (OH)	C2 (9) [3.15] - ASN283 (OD1)
H1 (35) [3.33] - ASN283 (OD1)	O2 (3) [3.68] - TYR803 (OH)	C14 (21) [3.64] - LEU287 (CD2, CG)	C4 (11) [3.56] - TYR803 (CE1)		C4 (11) [3.52] - ASN283 (OD1)
	O5 (6) [3.45] - TYR1076 (OH)	C24 (31) [3.45] - PHE806 (CD2, CE2)	C5 (12) [3.74] - TYR803 (CE1, CZ)		O3 (4) [3.46] - ILE286 (CG2)
	O4 (5) [3.35] - LYS1079 (NZ)	C14 (21) [3.82] - PHE806 (CE2)	C8 (15) [3.58] - TYR803 (CE1)		N1 (7) [3.44] - TYR803 (CE1)
		C26 (33) [3.44] - ALA807 (CB)	C10 (17) [3.47] - TYR803 (CE1)		C3 (10) [3.35] - TYR803 (OH)
		C27 (34) [3.51] - HIS913 (CD2)	C13 (20) [3.88] - TYR803 (CE1)		C5 (12) [2.99] - TYR803 (OH)
		C27 (34) [3.67] - PHE914 (CE1)			C7 (14) [3.68] - TYR803 (OH)
					C8 (15) [3.59] - TYR803 (OH)
					O2 (3) [3.81] - PHE806 (CD2)
					C27 (34) [3.68] - HIS913 (NE2)
					O5 (6) [3.87] - LEU917 (CD1)
					O5 (6) [3.29] - TYR1076 (CE1, CZ)
					C20 (27) [3.53] - TYR1076 (OH)
					C19 (26) [3.74] - TYR1076 (OH)
					C25 (32) [3.24] - LYS1079 (NZ)

PfMDR1-1075I

polar	hydrophobic	pi-pi	other
N1 (7) [3.55] - TYR803 (OH)	C22 (29) [3.70] - ILE286 (CG2)	C23 (30) [3.04] - TYR290 (CB, CD1, CD2, CE1, CG)	C26 (33) [3.05] - ASN283 (CB, CG, OD1)
O3 (4) [3.51] - TYR803 (OH)	C17 (24) [3.79] - ILE286 (CG2)	C22 (29) [3.50] - TYR290 (CB, CD2, CG)	O2 (3) [3.89] - ILE286 (CG2)
	C26 (33) [3.83] - ILE286 (CD1)	C8 (15) [3.89] - TYR803 (CZ)	O2 (3) [3.82] - LEU287 (CD2)
	C24 (31) [3.46] - LEU287 (CD2, CG)	C21 (28) [3.04] - TYR810 (CD1, CD2, CE1, CG, CZ)	O3 (4) [3.04] - TYR803 (CD1, CE1, CZ)
	C14 (21) [3.52] - TYR803 (CE1)	C23 (30) [3.28] - TYR810 (CD1, CE1, CZ)	C5 (12) [3.86] - TYR803 (OH)
	C15 (22) [3.46] - TYR803 (CE1, CZ)	C18 (25) [3.57] - PHE1072 (CE1)	C8 (15) [3.18] - TYR803 (OH)
	C24 (31) [3.56] - PHE806 (CD2, CE2)		C10 (17) [3.06] - TYR803 (OH)
	C27 (34) [3.37] - ILE1071 (CD1, CG2)		C14 (21) [3.38] - TYR803 (OH)
			C15 (22) [3.62] - TYR803 (OH)
			O3 (4) [3.32] - PHE806 (CD2, CE2)
			O2 (3) [3.67] - PHE806 (CE2)
			O5 (6) [3.29] - ILE1071 (CG2)
			O4 (5) [3.40] - PHE1072 (CE1)

PfMDR1-1072L

polar	hydrophobic	pi-pi	cation-pi	halogen-bond	other
O1 (2) [3.68] - TYR803 (OH)	C23 (30) [3.84] - ILE286 (CG2)	C16 (23) [3.73] - TYR803 (CE1, CZ)	H1 (35) [2.47] - PHE1068 (CB, CD2, CE2, CG)	Cl1 (1) [3.55] - TYR803 (OH)	O3 (4) [3.63] - LEU287 (CD2)
	C22 (29) [3.45] - ILE286 (CG2)	C21 (28) [3.32] - TYR803 (CE1, CZ)			Cl1 (1) [3.48] - TYR803 (CE1, CZ)
	C24 (31) [3.87] - TYR290 (CE2)	C4 (11) [3.61] - PHE1068 (CB, CD2, CG)			C3 (10) [3.77] - TYR803 (OH)
	C26 (33) [3.87] - TYR290 (CE2)	C10 (17) [3.61] - PHE1068 (CD2)			C6 (13) [3.03] - TYR803 (OH)
	C14 (21) [3.89] - ALA807 (CB)	C9 (16) [3.63] - PHE1068 (CD1)			C7 (14) [3.25] - TYR803 (OH)
	C14 (21) [3.15] - TYR810 (CB, CD2, CG)	C12 (19) [3.33] - PHE1068 (CD1, CE1)			C11 (18) [3.83] - TYR803 (OH)
	C15 (22) [3.68] - TYR810 (CD1, CD2, CG)	C18 (25) [3.46] - PHE1068 (CD1, CE1)			C16 (23) [3.38] - TYR803 (OH)
	C2 (9) [2.89] - PHE1068 (CB, CD1, CD2, CG)				C21 (28) [3.68] - TYR803 (OH)
	C14 (21) [3.59] - PHE1068 (CD2)				Cl1 (1) [3.66] - PHE806 (CD2)
	C25 (32) [3.67] - PHE1068 (CE1)				O3 (4) [3.29] - PHE806 (CD2, CE2)
	C19 (26) [3.49] - ILE1071 (CB, CD1, CG2)				H1 (35) [3.48] - ALA807 (CB)
	C13 (20) [3.80] - ILE1071 (CG2)				O3 (4) [3.55] - TYR810 (CB, CD1, CG)
	C27 (34) [3.66] - ILE1071 (CD1)				O2 (3) [3.35] - TYR810 (CD1, CD2, CE1, CE2, CG, CZ)
	C1 (8) [3.22] - LEU1072 (CD1, CD2, CG)				N1 (7) [3.00] - PHE1068 (CB, CD2, CE2, CG)
	C2 (9) [3.62] - LEU1072 (CD1, CG)				O4 (5) [3.49] - PHE1068 (CE1)
	C6 (13) [3.30] - LEU1072 (CD2)				

Atom names and their corresponding number in brackets corresponds to the numbering of HHQ atoms in Supplementary Figure 3. Numbers in the square brackets indicate the lowest interatomic distance between the given ligand atom and the amino acid residue.

Supplementary Table 11. Interactions of GNF-Pf-5668 with wild-type and mutated PfMDR1.

wild-type PfMDR1

hydrogen bonds	hydrophobic	pi-pi	other
O3 (*4*) [*3.38*] – TYR803 (*OH*)	C28 (*35*) [*3.27*] – ILE286 (*CB, CD1, CG2*)	C22 (*29*) [*3.77*] – TYR290 (*CG*)	O2 (*3*) [*3.89*] – ILE286 (*CG2*)
	C25 (*32*) [*3.45*] – LEU287 (*CD2, CG*)	C21 (*28*) [*3.26*] – PHE806 (*CD2, CE2*)	C15 (*22*) [*3.65*] – TYR803 (*OH*)
	C28 (*35*) [*3.76*] – LEU287 (*CG*)	C23 (*30*) [*3.47*] – PHE806 (*CE2*)	C24 (*31*) [*3.69*] – TYR803 (*OH*)
	C21 (*28*) [*3.71*] – LEU287 (*CD2*)	C21 (*28*) [*3.84*] – TYR810 (*CB, CG*)	O1 (*2*) [*3.65*] – PHE1068 (*CD2, CG*)
	C23 (*30*) [*3.67*] – LEU287 (*CD2*)	C23 (*30*) [*3.23*] – TYR810 (*CD1, CE1, CG*)	O5 (*6*) [*3.47*] – ILE1071 (*CG2*)
	C24 (*31*) [*3.25*] – TYR803 (*CE1, CZ*)	C12 (*19*) [*3.38*] – PHE1068 (*CD1, CE1*)	O4 (*5*) [*3.73*] – ILE1071 (*CD1*)
	C25 (*32*) [*3.31*] – PHE806 (*CD2, CE2*)	C9 (*16*) [*3.80*] – PHE1068 (*CE1*)	O1 (*2*) [*3.62*] – PHE1072 (*CZ*)
	C6 (*13*) [*3.22*] – PHE1068 (*CE1, CE2, CZ*)		
	C26 (*33*) [*3.82*] – PHE1068 (*CE1*)		
	C27 (*34*) [*3.42*] – ILE1071 (*CG2*)		
	C26 (*33*) [*3.37*] – ILE1071 (*CD1*)		

PfMDR1-290F

hydrogen bonds	polar	hydrophobic	pi-pi	other
N1 (*7*) [*3.15*] – ASN283 (*OD1*)	O2 (*3*) [*3.85*] – TYR803 (*OH*)	C14 (*21*) [*3.69*] – ILE286 (*CB, CG2*)	C19 (*26*) [*3.75*] – TYR1076 (*CE1, CZ*)	C26 (*33*) [*3.72*] – ASN283 (*ND2*)
O1 (*2*) [*2.94*] – TYR803 (*OH*)	O4 (*5*) [*3.77*] – HIS913 (*NE2*)	C10 (*17*) [*3.87*] – ILE286 (*CD1*)		C2 (*9*) [*3.49*] – ASN283 (*OD1*)
H1 (*36*) [*3.64*] – ASN283 (*OD1*)	O5 (*6*) [*3.62*] – HIS913 (*NE2*)	C14 (*21*) [*3.67*] – LEU287 (*CG*)		C4 (*11*) [*3.80*] – ASN283 (*OD1*)
H1 (*36*) [*3.55*] – ILE286 (*OD1*)	O5 (*6*) [*3.25*] – TYR1076 (*OH*)	C26 (*33*) [*3.38*] – TYR803 (*CB, CD1, CG*)		F1 (*1*) [*2.68*] – ILE286 (*CD1, CG1, CG2*)
		C3 (*10*) [*3.76*] – TYR803 (*CE1*)		O3 (*4*) [*3.84*] – ILE286 (*CG2*)
		C4 (*11*) [*3.83*] – TYR803 (*CE1*)		N1 (*7*) [*3.58*] – ILE286 (*CD1*)
		C8 (*15*) [*3.86*] – TYR803 (*CE1*)		O3 (*4*) [*3.87*] – LEU287 (*CD2*)
		C24 (*31*) [*3.39*] – TYR803 (*CE1, CZ*)		O2 (*3*) [*3.70*] – TYR803 (*CE1*)
		C15 (*22*) [*3.78*] – PHE806 (*CE2*)		C3 (*10*) [*3.29*] – TYR803 (*OH*)
		C27 (*34*) [*3.46*] – HIS913 (*CD2*)		C5 (*12*) [*3.13*] – TYR803 (*OH*)
		C27 (*34*) [*3.80*] – LEU917 (*CD1*)		C7 (*14*) [*3.32*] – TYR803 (*OH*)
		C28 (*35*) [*3.64*] – PHE1072 (*CE2, CZ*)		C8 (*15*) [*3.85*] – TYR803 (*OH*)
				C24 (*31*) [*3.16*] – TYR803 (*OH*)
				O2 (*3*) [*3.28*] – PHE806 (*CD2, CE2*)
				O3 (*4*) [*3.68*] – PHE806 (*CE2*)
				C27 (*34*) [*3.46*] – HIS913 (*NE2*)
				O5 (*6*) [*3.47*] – LEU917 (*CD1*)
				O5 (*6*) [*3.45*] – TYR1076 (*CE1, CZ*)
				C20 (*27*) [*3.33*] – TYR1076 (*OH*)
				C19 (*26*) [*3.17*] – TYR1076 (*OH*)

PfMDR1-1072L

hydrophobic	pi-pi	cation-pi	other
C21 (*28*) [*3.27*] – ILE286 (*CD1, CG2*)	C22 (*29*) [*3.24*] – TYR803 (*CE1, CZ*)	H1 (*36*) [*3.33*] – PHE1068 (*CB, CD2, CG*)	O3 (*4*) [*3.80*] – LEU287 (*CD2*)
C23 (*30*) [*3.85*] – ILE286 (*CG2*)	C17 (*24*) [*3.64*] – TYR803 (*CE1, CZ*)		C1 (*8*) [*3.77*] – TYR803 (*OH*)
C24 (*31*) [*3.56*] – TYR290 (*CD2, CE2, CZ*)	C9 (*16*) [*3.77*] – PHE1068 (*CD1*)		C2 (*9*) [*3.81*] – TYR803 (*OH*)
C14 (*21*) [*3.64*] – ALA807 (*CB*)	C12 (*19*) [*3.82*] – PHE1068 (*CD1*)		C3 (*10*) [*3.51*] – TYR803 (*OH*)
C14 (*21*) [*3.31*] – TYR810 (*CB, CD2, CG*)	C18 (*25*) [*3.57*] – PHE1068 (*CD1, CE1*)		C4 (*11*) [*3.75*] – TYR803 (*OH*)
C15 (*22*) [*3.87*] – TYR810 (*CD1, CG*)	C20 (*27*) [*3.57*] – PHE1068 (*CD1, CE1*)		C6 (*13*) [*2.96*] – TYR803 (*OH*)
C2 (*9*) [*3.56*] – PHE1068 (*CB, CG*)	C19 (*26*) [*3.46*] – PHE1068 (*CD1*)		C7 (*14*) [*3.28*] – TYR803 (*OH*)
C27 (*34*) [*3.33*] – PHE1068 (*CD1, CE1*)	C13 (*20*) [*3.57*] – PHE1068 (*CD1*)		C11 (*18*) [*3.85*] – TYR803 (*OH*)
C28 (*35*) [*3.90*] – PHE1068 (*CE2*)			C22 (*29*) [*3.76*] – TYR803 (*OH*)
C19 (*26*) [*3.40*] – ILE1071 (*CB, CD1, CG2*)			C17 (*24*) [*3.40*] – TYR803 (*OH*)
C27 (*34*) [*3.64*] – ILE1071 (*CD1*)			O3 (*4*) [*3.58*] – PHE806 (*CE2*)
C1 (*8*) [*3.20*] – LEU1072 (*CD2, CG*)			N1 (*7*) [*3.79*] – ALA807 (*CB*)
C2 (*9*) [*3.45*] – LEU1072 (*CD1, CD2, CG*)			H1 (*36*) [*3.13*] – ALA807 (*CB*)
C6 (*13*) [*3.68*] – LEU1072 (*CD2*)			O3 (*4*) [*3.39*] – TYR810 (*CD1, CE1, CG*)
			O2 (*3*) [*3.50*] – TYR810 (*CD2, CE1, CE2, CZ*)
			N1 (*7*) [*3.83*] – PHE1068 (*CD2*)
			O4 (*5*) [*3.90*] – PHE1068 (*CE1*)
			O5 (*6*) [*3.86*] – ILE1071 (*CD1*)

PfMDR1-1075I

hydrogen bonds	hydrophobic	pi-pi	halogen-bond	other
O3 (*4*) [*3.13*] – TYR803 (*OH*)	C28 (*35*) [*3.47*] – LEU287 (*CD1, CG*)	C21 (*28*) [*3.43*] – PHE806 (*CD2, CE2*)	F1 (*1*) [*3.72*] – TYR803 (*OH*)	C28 (*35*) [*3.33*] – ASN283 (*CB, CG, OD1*)
	C23 (*30*) [*3.59*] – LEU287 (*CD2*)	C23 (*30*) [*3.24*] – PHE806 (*CD2, CE2*)		C25 (*32*) [*3.80*] – ASN283 (*OD1*)
	C6 (*13*) [*3.02*] – PHE1068 (*CD1, CD2, CE1, CE2, CZ*)	C21 (*28*) [*3.84*] – TYR810 (*CB*)		O2 (*3*) [*3.46*] – ILE286 (*CG2*)
	C1 (*8*) [*3.83*] – PHE1068 (*CE1, CZ*)	C23 (*30*) [*3.30*] – TYR810 (*CB, CD1, CG*)		C15 (*22*) [*3.76*] – TYR803 (*OH*)
	C27 (*34*) [*3.44*] – ILE1071 (*CD1*)	C22 (*29*) [*3.67*] – TYR810 (*CD1, CE1*)		O1 (*2*) [*3.61*] – PHE1068 (*CD2, CG*)
	C26 (*33*) [*3.68*] – PHE1072 (*CE1*)	C9 (*16*) [*3.79*] – PHE1068 (*CE1*)		O1 (*2*) [*3.37*] – PHE1072 (*CE2, CZ*)
				F1 (*1*) [*3.86*] – PHE1072 (*CE2*)
				O4 (*5*) [*3.37*] – PHE1072 (*CE1*)

Atom names and their corresponding number in brackets corresponds to the numbering of HHQ atoms in Supplementary Figure 3. Numbers in the square brackets indicate the lowest interatomic distance between the given ligand atom and the amino acid residue.

Supplementary Table 12. Compound interaction table.

	GNF-Pf-5640				GNF-Pf-5660						GNF-Pf-5668					
	hydrogen bonds	polar	hydro-phobic	pi-pi	hydrogen bonds	polar	hydro-phobic	pi-pi	cation-pi	halogen-bond	hydrogen bonds	polar	hydro-phobic	pi-pi	cation-pi	halogen-bond
wild-type PfMDR1	N1 H1		C1 C2 C26 C27 C28	C13 C19 C22 C24		O2 O5	C14 C24 C26 C27 C7	C13 C18 C19 C20 C21 C22 C23			O3		C21 C23 C24 C25 C26 C27 C28 C6	C12 C21 C22 C23 C9		
PfMDR1-290F		O4	C1 C16 C21 C24 C25 C26 C27 C6 C7	C13 C17 C19 C22	N1 H1	O1 O2 O4 O5	C17 C14 C24 C26 C27	C3 C10 C13 C4 C5 C8		Cl1	N1 O1 H1	O2 O4 O5	C10 C14 C15 C24 C26 C27 C28 C3 C4 C8	C19		
PfMDR1-1072L		O1	C1 C15 C21 C25 C26 C27 C28 C6 C7	C19 C22 C24		O1	C23 C1 C13 C14 C15 C19 C2 C22 C24 C25 C26 C27 C6	C16 C10 C12 C18 C21 C4 C9	H1	Cl1			C21 C1 C14 C15 C19 C2 C23 C24 C27 C28 C6	C22 C12 C13 C17 C18 C19 C20 C9	H1	
PfMDR1-1075I			C1 C19 C21 C24 C25 C26 C27 C6 C7	C22 C24 C9		N1 O3	C22 C14 C15 C17 C24 C26 C27	C18 C21 C22 C23 C8			O3		C28 C23 C6 C1 C27 C26	C21 C23 C21 C23 C22 C9		F1

Supplementary Table 13a. IC_{50} data for common and experimental antimalarials for the edited F1072L and S1075I lines and the selected Y290F and F1072L + CNV lines in the Dd2-B2 background.

	Dd2-B2			Dd2-B2 F1072L edited					Dd2-B2 S1075I edited					Dd2-B2 Y290F selected (5660-2B6H1)					Dd2-B2 F1072L + CNV selected (5668-3C4D2)				
Antimalarial	Mean IC_{50}	SEM	n	Mean IC_{50}	SEM	n	Fold shift vs Dd2-B2	*P*-value vs Dd2-B2	Mean IC_{50}	SEM	n	Fold shift vs Dd2-B2	*P*-value vs Dd2-B2	Mean IC_{50}	SEM	n	Fold shift vs Dd2-B2	*P*-value vs Dd2-B2	Mean IC_{50}	SEM	n	Fold shift vs Dd2-B2	*P*-value vs Dd2-B2
Chloroquine	327.5	28.4	5	394.8	27.8	5	1.2	0.4762	397.2	32.7	5	1.2	0.4352	299.1	46.5	5	0.9	>0.9999	n.d.	n.d.	n.d.	n.d.	n.d.
Dihydroartemisin	1.9	0.1	7	1.1	0.1	5	0.6	9.57E-05	1.2	0.1	4	0.6	0.0015	1.1	0.2	3	0.6	0.0020	1.4	0.1	3	0.7	0.0306
Quinine	185.5	6.6	9	52.0	2.5	5	0.3	2.22E-08	36.2	2.1	5	0.2	5.82E-09	49.6	3.1	4	0.3	1.79E-07	109.6	6.1	4	0.6	9.0E-05
Lumefantrine	1.9	0.2	10	0.6	0.1	5	0.3	0.0003	2.3	0.1	4	1.2	>0.9999	1.0	0.1	5	0.5	0.0073	1.4	0.1	4	0.7	0.2438
Monodesethyl-Amodiaquine	43.9	4.3	5	60.1	5.1	5	1.4	0.0658	53.0	4.2	5	1.2	0.5455	46.1	6.7	4	1.0	>0.9999	n.d.	n.d.	n.d.	n.d.	n.d.
Ferroquine	10.8	0.9	6	12.9	1.4	5	1.2	0.4130	11.8	0.9	5	1.1	>0.9999	10.8	1.2	4	1.0	>0.9999	n.d.	n.d.	n.d.	n.d.	n.d.
Mefloquine	17.0	0.9	12	4.7	0.3	6	0.3	1.61E-07	12.4	0.8	5	0.7	0.0258	8.5	0.2	4	0.5	0.000297	12.1	0.1	4	0.7	0.0253
ACT-451840	0.7	0.0	10	11.1	0.8	4	16.1	1.06E-10	3.8	0.1	5	5.5	3.1E-13	31.0	4.4	3	45.0	8.2E-08	10.8	0.9	3	15.7	4.2E-10
Piperaquine	12.0	1.0	11	16.4	1.1	6	1.4	0.0525	14.9	0.8	5	1.2	0.3319	14.6	0.9	5	1.2	0.5453	9.4	1.0	4	0.8	0.6849
Monodesethyl-Chloroquine	651.0	89.5	4	711.4	101.9	4	1.1	>0.9999	758.1	93.8	4	1.2	>0.9999	593.0	51.3	4	0.9	0.9435	n.d.	n.d.	n.d.	n.d.	n.d.

SEM: standard error of the mean; n: number of repeated experiments; *P* value vs Dd2-B2: Welch's t-test corrected for multiple comparisons using the Bonferroni-Dunn method; n.d.: not done. All values are given in nM.

Supplementary Table 13b. IC_{50} data for the NF54 and selected NF54 Y290F lines.

	NF54			NF54 Y290F selected				
Antimalarial	Mean IC_{50}	SEM	n	Mean IC_{50}	SEM	n	Fold shift vs NF54	*P* value vs NF54
Dihydroartemisin	1.4	0.1	5	1.5	0.2	4	1.1	0.7455
Quinine	35.1	1.6	5	18.7	1.6	4	0.5	0.0002
Lumefantrine	2.9	0.2	4	3.4	0.3	4	1.2	0.2005
Mefloquine	17.7	0.4	5	17.5	1.2	4	1.0	0.8843
ACT-451840	0.5	0.0	4	74.3	4.9	4	165.1	0.0006
Piperaquine	7.6	0.7	5	9.3	0.9	4	1.2	0.1821

SEM: standard error of the mean; n: number of repeated experiments; *P* value vs Dd2-B2: Welch's t-test. All values are given in nM.

Supplementary Table 13c. IC_{50} data for the FCB and $KD1^{mdr1}$ lines.

	FCB			$KD1^{mdr1}$				
Antimalarial	Mean IC_{50}	SEM	n	Mean IC_{50}	SEM	n	Fold shift vs FCB	*P* value vs FCB
Lumefantrine	1.3	0.1	4	0.9	0.1	5	0.7	0.0383
Mefloquine	10.7	0.5	5	8.9	0.7	5	0.8	0.0720
Piperaquine	10.4	0.4	4	11.5	1.4	4	1.1	0.4706

SEM: standard error of the mean; n: number of repeated experiments; *P* value vs Dd2-B2: Welch's t-test. All values are given in nM.

Supplementary Table 14a. IC_{90} data for common and experimental antimalarials for the edited F1072L and S1075I lines and the selected Y290F and F1072L + CNV lines in the Dd2-B2 background.

	Dd2-B2			Dd2-B2 F1072L edited					Dd2-B2 S1075I edited					Dd2-B2 Y290F selected (5660-2B6H1)					Dd2-B2 F1072L + CNV selected (5668-3C4D2)				
Antimalarial	Mean IC_{90}	SEM	n	Mean IC_{90}	SEM	n	Fold shift vs Dd2-B2	*P* value vs Dd2-B2	Mean IC_{90}	SEM	n	Fold shift vs Dd2-B2	*P* value vs Dd2-B2	Mean IC_{90}	SEM	n	Fold shift vs Dd2-B2	*P* value vs Dd2-B2	Mean IC_{50}	SEM	n	Fold shift vs Dd2-B2	*P* value vs Dd2-B2
Chloroquine	492.4	49.8	5	700.0	86.8	5	1.4	0.0783	606.2	53.6	5	1.2	0.6183	493.3	71.8	5	1.0	>0.9999	n.d.	n.d.	n.d.	n.d.	n.d.
Dihydroartemisin	3.7	0.1	7	2.6	0.1	5	0.7	0.0004	2.9	0.4	4	0.8	0.1585	2.3	0.1	3	0.7	0.0009	2.7	0.3	3	0.7	0.0249
Quinine	411.4	17.5	9	123.4	10.1	5	0.3	3.061E-07	79.0	5.1	5	0.2	4.251E-08	118.9	11.2	4	0.3	1.797E-06	235.7	7.9	4	0.6	0.0002
Lumefantrine	5.6	0.9	10	2.5	0.2	5	0.3	0.1015	9.6	1.4	4	1.4	0.1173765	6.7	0.7	5	0.9	>0.9999	3.9	0.5	4	0.7	0.9813
Monodesethyl-Amodiaquine	58.2	5.9	5	89.0	8.7	5	1.5	0.0134	67.4	7.0	5	1.2	>0.9999	60.9	8.7	4	1.0	>0.9999	n.d.	n.d.	n.d.	n.d.	n.d.
Ferroquine	13.2	1.3	6	16.9	1.5	5	1.3	0.1470	14.5	1.1	5	1.1	>0.9999	14.1	0.7	4	1.1	>0.9999	n.d.	n.d.	n.d.	n.d.	n.d.
Mefloquine	39.8	1.5	12	11.2	0.7	6	0.3	0.0448	34.2	2.8	5	0.9	0.3025034	22.5	1.0	4	0.6	6.908E-05	25.7	1.2	4	0.6	0.0006
ACT-451840	1.1	0.1	10	42.9	2.1	4	37.1	1.135E-12	13.3	1.0	5	11.5	5.647E-10	134.5	30.6	3	116.4	8.691E-06	41.7	9.8	3	38.1	1.429E-05
Piperaquine	19.4	2.1	11	28.8	1.7	6	1.3	0.0349	25.3	1.6	6	1.1	0.2916	25.8	1.8	4	1.1	0.3552	15.1	2.0	4	0.8	>0.9999
Monodesethyl-Chloroquine	1007.3	103.7	4	1270.8	146.3	4	1.3	0.3953	1229.4	153.0	4	1.2	0.5995	1098.4	80.4	4	1.1	>0.9999	n.d.	n.d.	n.d.	n.d.	n.d.

SEM: standard error of the mean; n: number of repeated experiments; P value vs Dd2-B2: Welch's t-test corrected for multiple comparisons using the Bonferroni-Dunn method; n.d.: not done. All values are given in nM.

Supplementary Table 14b. IC_{90} data for the NF54 and selected NF54 Y290F lines.

	NF54			NF54 Y290F selected				
Antimalarial	Mean IC_{90}	SEM	n	Mean IC_{90}	SEM	n	Fold shift vs NF54	*P* value vs Dd2-B2
Dihydroartemisin	3.2	0.3	5	3.8	0.7	4	1.2	0.4436
Quinine	55.2	2.5	5	29.7	2.9	4	0.5	0.0004
Lumefantrine	7.5	0.7	4	11.5	2.9	4	1.5	0.2570
Mefloquine	29.7	1.6	5	28.7	0.7	4	1.0	0.5979
ACT-451840	0.6	0.1	4	163.9	13.3	4	253.7	0.0012
Piperaquine	9.9	1.6	5	13.0	1.4	4	1.3	0.1877

SEM: standard error of the mean; n: number of repeated experiments; *P* value vs Dd2-B2: Welch's t-test. All values are given in nM.

Supplementary Table 14c. IC_{90} data for the FCB and $KD1^{mdr1}$ lines.

	FCB			$KD1^{mdr1}$				
Antimalarial	Mean IC_{90}	SEM	n	Mean IC_{90}	SEM	n	Fold shift vs FCB	*P* value vs Dd2-B2
Lumefantrine	11.0	3.7	4	3.0	3.0	5	0.3	0.1200
Mefloquine	29.9	3.2	5	21.4	1.6	5	0.7	0.0568
Piperaquine	17.0	1.0	4	20.6	2.9	4	1.2	0.3125

SEM: standard error of the mean; n: number of repeated experiments; *P* value vs Dd2-B2: Welch's t-test. All values are given in nM.

Supplementary Table 15. Heme fractionation data.

		Dd2-B2												PfMDR1^{selY290F}											
	drug pressure	GNF-Pf-5660				MQ				LMF				GNF-Pf-5660				MQ				LMF			
		Mean	SEM	n	*P* value	Mean	SEM	*P* value	n	Mean	SEM	n	*P* value	Mean	SEM	n	*P* value	Mean	SEM	n	*P* value	Mean	SEM	n	*P* value
Hemoglobin	0.00x IC_{50}	1.74	0.23	7	-	2.10	0.82	-	4	2.10	0.82	4	-	4.26	0.16	6	-	2.41	0.08	5	-	1.42	0.12	8	-
	0.10x IC_{50}	1.55	0.25	7	0.5896	1.36	0.15	0.3009	6	1.27	0.25	4	0.3711	3.28	0.43	6	0.0573	2.63	0.64	6	0.7670	1.95	0.46	7	0.2585
	0.25x IC_{50}	1.57	0.23	7	0.6245	1.21	0.12	0.2651	5	2.02	0.76	4	0.9436	2.75	0.25	6	0.0005	3.89	0.93	7	0.2172	1.73	0.17	6	0.1488
	0.50x IC_{50}	1.67	0.25	7	0.8505	1.80	0.15	0.6689	6	2.52	0.64	8	0.7097	3.59	0.72	6	0.3821	2.63	0.85	7	0.8315	1.88	0.24	6	0.0861
	1.00x IC_{50}	1.29	0.23	7	0.1971	2.12	0.22	0.9802	6	2.80	0.59	8	0.5093	3.09	0.59	6	0.0855	2.39	0.29	6	0.9648	2.97	0.62	8	0.0280
	2.00x IC_{50}	2.64	1.09	7	0.4316	2.25	0.45	0.8680	5	2.96	0.55	8	0.3940	2.71	0.57	5	0.0190	2.42	0.48	8	0.9834	2.40	0.40	8	0.0362
Heme	0.00x IC_{50}	4.84	0.22	7	-	4.90	0.83	-	4	4.90	0.83	4	-	4.87	0.63	6	-	6.84	0.58	5	-	4.92	0.37	8	-
	0.10x IC_{50}	4.14	0.37	7	0.1296	3.70	0.42	0.1901	6	5.06	0.44	4	0.8703	4.71	0.44	6	0.8391	5.57	0.62	6	0.1718	5.36	0.29	7	0.3754
	0.25x IC_{50}	4.24	0.70	6	0.3972	3.81	0.40	0.2206	6	5.49	0.64	4	0.5915	4.86	0.52	6	0.9905	8.00	0.68	7	0.2516	6.27	0.27	6	0.0166
	0.50x IC_{50}	3.88	0.45	7	0.0766	3.84	0.32	0.2345	5	5.27	0.53	8	0.7082	6.15	0.69	6	0.2007	5.93	0.49	7	0.2551	5.79	0.36	6	0.1232
	1.00x IC_{50}	5.08	0.24	7	0.4793	3.46	0.54	0.1633	6	5.02	0.25	8	0.8662	6.87	1.16	6	0.1591	5.04	0.29	6	0.0158	5.63	0.68	8	0.3730
	2.00x IC_{50}	4.32	0.41	7	0.2886	3.28	0.57	0.1605	4	5.64	0.61	8	0.4950	5.93	1.80	5	0.5639	5.20	0.82	8	0.1800	5.68	0.59	8	0.2918
Hemozoin	0.00x IC_{50}	67.64	5.31	7	-	66.76	8.22	-	4	66.77	8.22	4	-	54.35	3.01	6	-	79.85	3.77	5	-	68.07	4.95	8	-
	0.10x IC_{50}	44.88	3.29	7	0.0034	65.63	4.22	0.8957	6	69.28	5.70	4	0.8102	50.65	5.93	6	0.5898	58.37	4.48	6	0.0059	69.65	8.04	7	0.8660
	0.25x IC_{50}	30.59	3.34	6	0.0001	72.72	3.79	0.4803	6	71.38	11.47	4	0.7547	50.12	7.07	6	0.5941	68.30	3.31	7	0.0454	70.09	8.84	6	0.8348
	0.50x IC_{50}	29.63	4.07	7	0.0001	75.56	5.49	0.3795	6	70.76	5.52	8	0.6893	49.93	3.56	6	0.3654	54.28	5.57	7	0.0026	59.25	6.39	6	0.2888
	1.00x IC_{50}	29.70	3.65	7	<0.0001	57.79	7.44	0.4519	6	61.99	3.77	8	0.5521	33.15	2.94	6	0.0005	46.02	2.85	6	<0.0001	47.60	3.25	8	0.0038
	2.00x IC_{50}	26.82	3.59	7	<0.0001	41.32	4.97	0.0274	5	67.00	4.28	8	0.9784	28.55	2.34	5	0.0001	32.92	3.31	8	<0.0001	30.47	3.46	8	<0.0001
Total heme iron	0.00x IC_{50}	74.21	5.30	7	-	73.76	9.05	-	4	73.76	9.05	4	-	63.48	3.59	6	-	89.10	4.39	5	-	74.40	5.06	8	-
	0.10x IC_{50}	55.15	5.53	8	0.0282	70.69	4.57	0.7457	6	75.61	5.86	4	0.8699	58.64	6.39	6	0.5234	66.56	4.96	6	0.0088	76.96	8.06	7	0.7869
	0.25x IC_{50}	36.48	3.41	6	0.0001	80.45	3.38	0.4442	6	78.89	12.83	4	0.7551	57.73	7.39	6	0.4993	80.19	3.39	7	0.1333	78.09	8.89	6	0.7083
	0.50x IC_{50}	35.18	3.90	7	<0.0001	83.48	5.68	0.3633	6	78.54	6.41	8	0.6757	59.67	3.63	6	0.4725	62.84	6.25	7	0.0103	66.92	6.60	6	0.3772
	1.00x IC_{50}	36.07	3.86	7	<0.0001	63.36	8.10	0.4257	6	69.81	3.80	8	0.6395	43.11	3.46	6	0.0022	53.45	3.31	6	<0.0001	56.20	3.59	8	0.0109
	2.00x IC_{50}	33.78	4.04	7	<0.0001	50.14	7.96	0.0904	5	75.60	4.87	8	0.8472	37.19	2.72	5	0.0003	40.54	4.17	8	<0.0001	38.55	3.48	8	<0.0001

Hemoglobin, free heme, hemozoin and total heme iron content per cell at different exposure levels to different drugs are expressed in fg/cell. The IC_{50} of Dd2-B2 and PfMDR1^{selY290F} to GNF-Pf-5660 is 23.7 nM and 3750 nM, respectively. The IC_{50} of Dd2-B2 and PfMDR1^{selY290F} to MQ is 24.2 nM and 8.5 nM, respectively. The IC_{50} of Dd2-B2 and PfMDR1^{selY290F} to LMF is 3.8 nM and 1.0 nM, respectively. *P* values were calculated by a two-tailed t-test. MQ: mefloquine; LMF: lumefantrine; SEM: standard error of the mean.

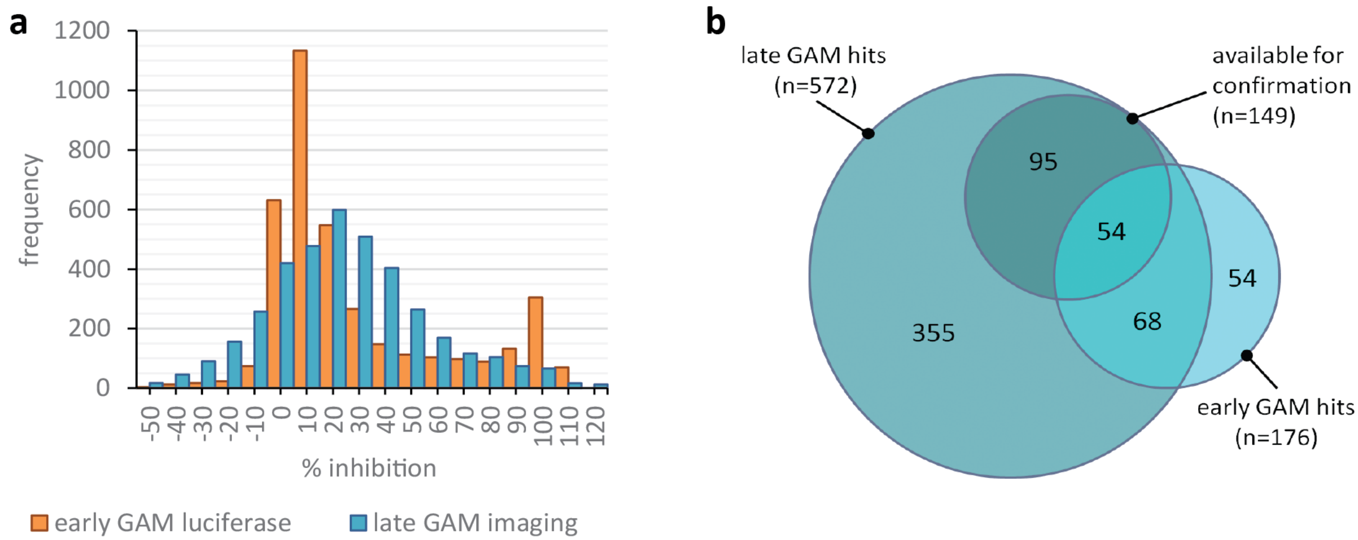
Supplementary Table 16. Western Blot data.

		4-day Peters test			
		mean IC_{50}	SEM	n	*P* value
PfMDR1$^{\text{wild-type Dd2-B2}}$	control	1.000	0.000	6	-
	HHQ	0.679	0.033	4	0.00034
	WR	1.223	0.089	3	0.33076
	MQ	0.936	0.201	4	>0.9999
	LMF	0.907	0.078	4	>0.9999
	CQ	1.144	0.383	4	>0.9999
PfMDR1$^{\text{selY290F}}$	control	1.000	0.000	4	-
	HHQ	1.106	0.194	4	0.6542

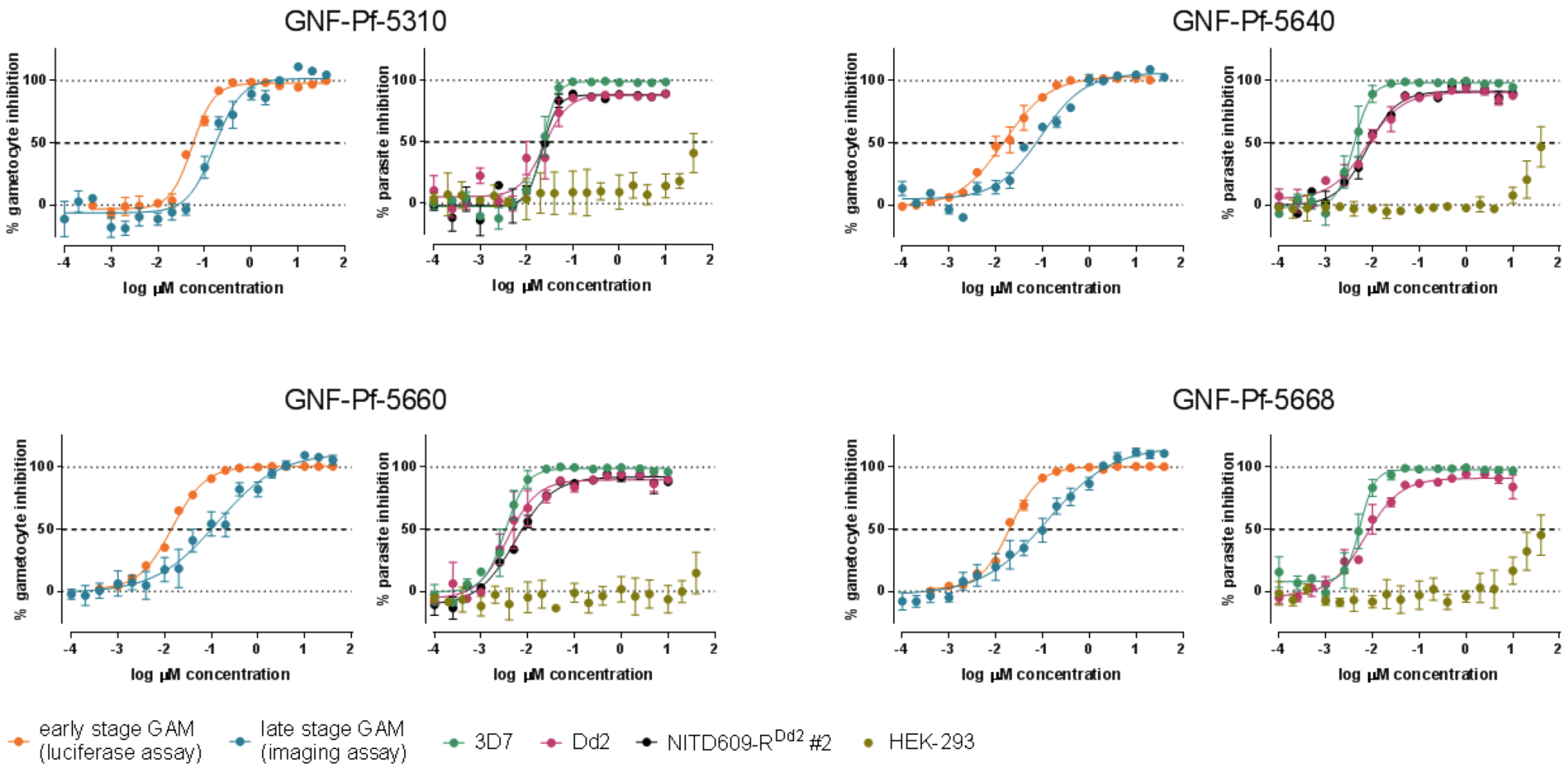
P values were calculated by a Welch's corrected t-test adjusted for multiple comparisons with the Bonferroni-Dunn method. HHQ: hexahydroquinoline GNF-Pf-5660; WR: WR99210; MQ: mefloquine; LMF: lumefantrin; SEM: standard error of the mean.

Supplementary Table 17. Parasite lines generated in this study.

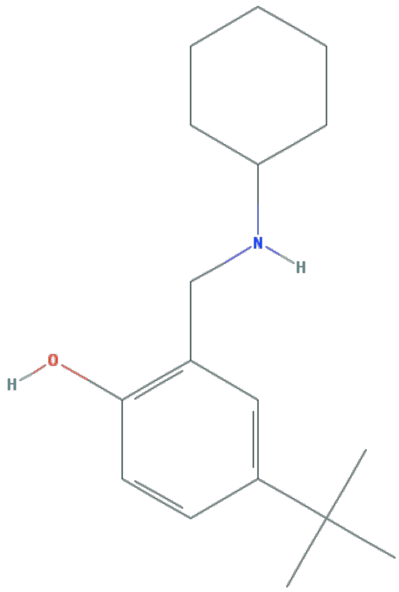
Parasite line	Alternative name	Origin	*pfmdr1* copy number	PfMDR1 mutation (allele balance)
5668-3C4D2	selected F1072L + CNV in Dd2-B2	GNF-Pf-5668-selected	4	F1072L (69%)
F1072L edited	$\text{PfMDR1}^{\text{edF1072L}}$	CRISPR/Cas9-edited	2	F1072L (100%)
5640-2C4C3	-	GNF-Pf-5640-selected	2	S1075I (100%)
S1075I edited	$\text{PfMDR1}^{\text{edS1075I}}$	CRISPR/Cas9-edited	2	S1075I (100%)
5640-3H9C7	-	GNF-Pf-5640-selected	2	Y290F (50%)
5660-2B6H1	$\text{PfMDR1}^{\text{selY290F}}$	GNF-Pf-5660-selected	2	Y290F (100%)



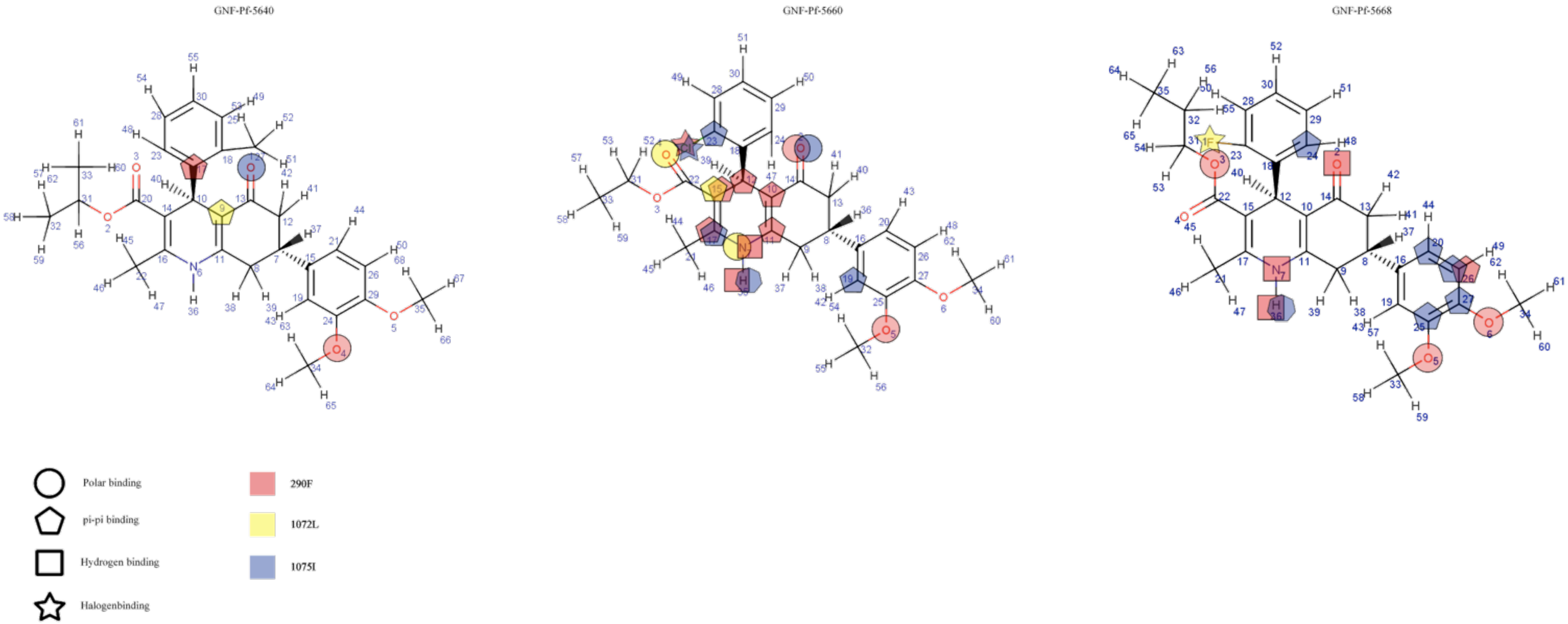
Supplementary Figure 1. Inhibitory activity distribution in the two primary screening campaigns **(a)** and availability of samples for dose-response confirmation testing in relationship to hit activity profile **(b)**.



Supplementary Figure 2. Gametocytocidal, asexual and cytotoxic dose-responses of the selected compounds. Error bars indicate standard error of the mean.

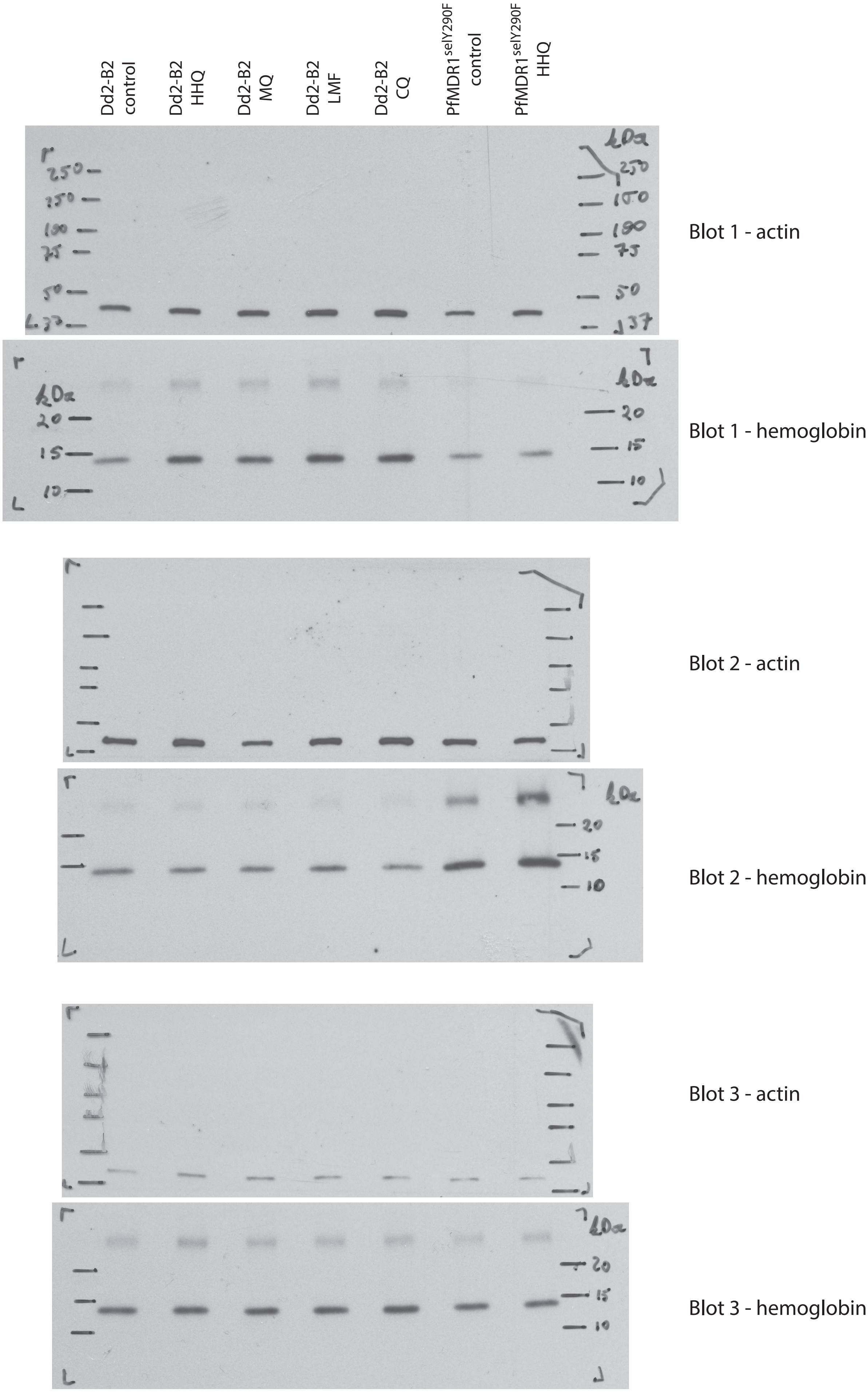


Supplementary Figure 3. Structure of GNF-Pf-5310.



Supplementary Figure 4. Moieties of HHQs that interact with mutant PfMDR1. Numbering is consistent with Supplementary Table 8.

Supplementary Figure 5a. Western blots experiments 1–3.



Supplementary Figure 5b. Western blots experiments 4-6.