SUPPLEMENTARY MATERIALS

Genetic mapping targets of differential chemical phenotypes in *Plasmodium falciparum*

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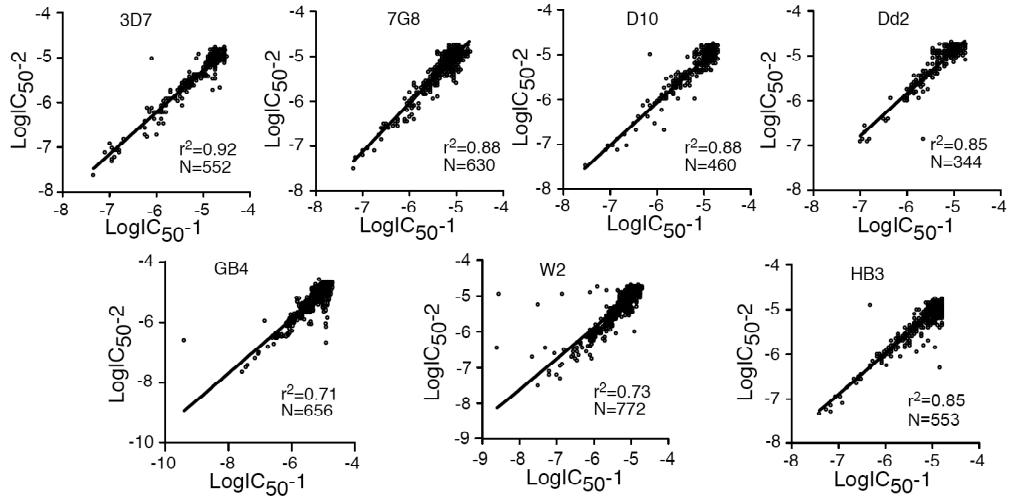
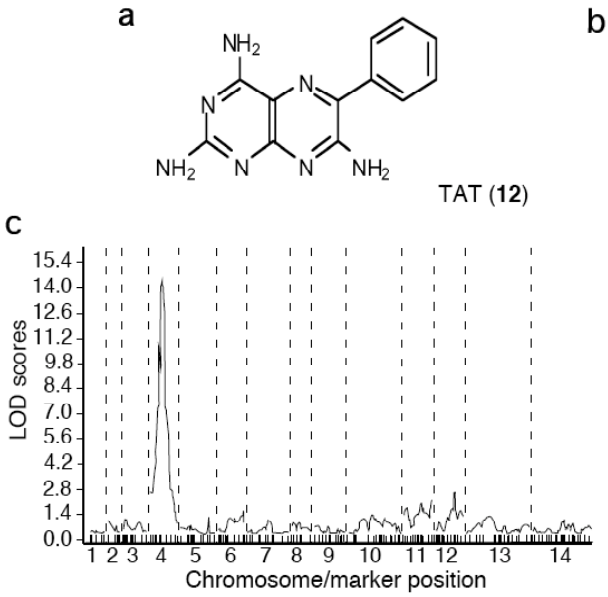
Supplementary Results:



Supplementary Figure 1 Correlation plots of potencies of active and inconclusive compounds between replicates. The solid line indicates the best fit of the data using linear regression analysis. $LogIC_{50}$-1 and $LogIC_{50}$-2 represent data from two replicates.

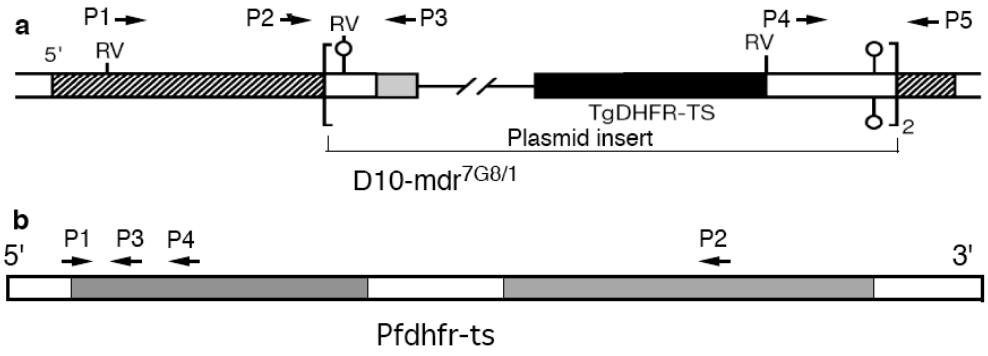


b

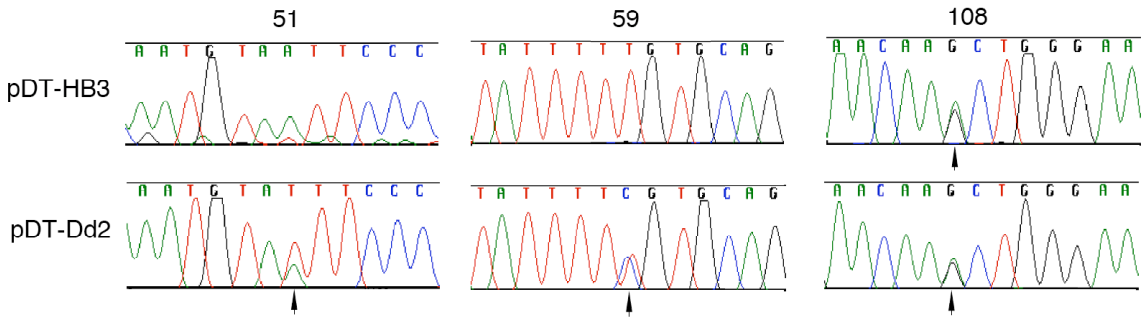
Parasite	IC_{50}(nm)	SD(nM)	C4M40	C4M19	C4M69	C3M35
			697*	738*	775*	797*
JB8	70393	4465	7G8	7G8	7G8	7G8
WE2	61785	1264	7G8	7G8	7G8	7G8
DAN	59746	15708	GB4	GB4	7G8	7G8
KC2	50695	12661	7G8	7G8	7G8	7G8
AL2	48456	6627	7G8	7G8	7G8	7G8
DEV	48211	4540	7G8	7G8	7G8	7G8
JF6	48189	8824	7G8	7G8	7G8	7G8
KC5	44582	3210	7G8	7G8	7G8	7G8
XB3	43831	9691	7G8	7G8	7G8	7G8
KB8	41742	0	7G8	7G8	7G8	7G8
JB12	40053	252	7G8	7G8	7G8	7G8
XD8	39806	9919	7G8	7G8	7G8	7G8
JC9	39642	1524	7G8	7G8	7G8	7G8
NF10	39605	1923	7G8	7G8	7G8	7G8
AUD	38539	2481	7G8	7G8	7G8	GB4
7G8	36531	1249	7G8	7G8	7G8	7G8
WC4	32669	4016	7G8	7G8	7G8	7G8
D2	29852	3577	7G8	7G8	7G8	7G8
LC12	24165	4737	7G8	7G8	7G8	GB4
XG10	3311	129	GB4	GB4	GB4	GB4
WF12	2657	62	GB4	GB4	GB4	GB4
GB4	2499	244	GB4	GB4	GB4	GB4
NIC	2303	235	7G8	GB4	GB4	GB4
XF12	2273	532	GB4	GB4	GB4	GB4
QF5	2155	209	GB4	GB4	GB4	GB4
KA6	2108	221	GB4	GB4	GB4	GB4
JON	2071	62	GB4	GB4	GB4	GB4
JC3	2032	92	GB4	GB4	GB4	GB4
JE11	1640	32	GB4	GB4	GB4	GB4
JH6	1503	124	GB4	GB4	GB4	7G8
KH7	1412	1008	GB4	GB4	GB4	GB4
LA10	1183	19	GB4	GB4	GB4	GB4
TF1	1096	375	GB4	GB4	GB4	GB4
XE7	855	59	GB4	GB4	GB4	GB4

*these numbers indicate chromosomal locations (kb) of the microsatellites.

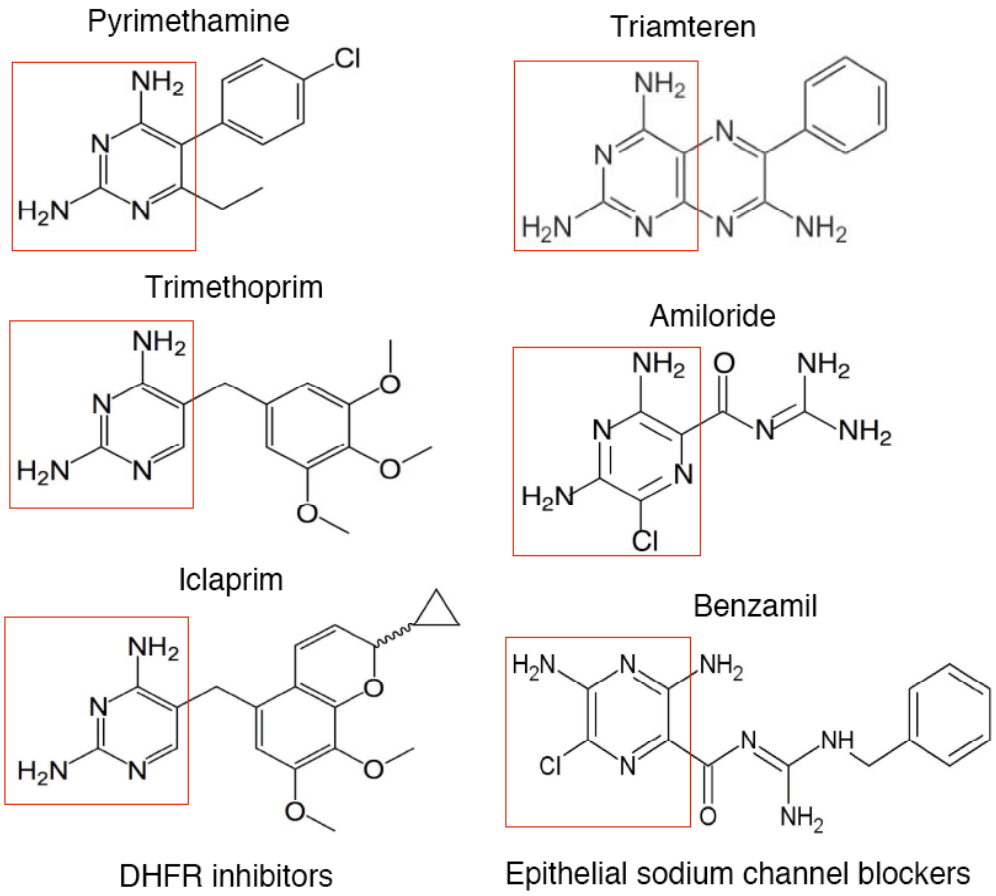
Supplementary Figure 2 Identification of genetic loci linked to response to triamterene (TAT). (**a**) Chemical structure of TAT. (**b**) TAT IC_{50} values (mean and standard deviation) and allelic designations for four microsatellite markers on chromosome 4 are shown for the parents and progeny of a GB4x7G8 cross. (**c**) Peaks of quantitative trait loci analysis linked to differential TAT responses are shown. Predicted genes within the chromosome 4 locus can be found in **Supplementary Table 9**.



Supplementary Figure 3. Diagram of the genes encoding *Plasmodium falciparum* homolog of the human P-glycoprotein (*pfmdr1)* and dihydrofolate reductase (*pfdhfr*) showing location of PCR primers used to amplify and sequence substitution sites in the gene. (**a**) Primers (arrows) for the amplification of polymorphic sites in *pfmdr1*. The drawing was adapted from Reed *et al* Nature 403, 906-909 (2002); (**b**) Primers (arrows) for amplifying and sequencing *pfdhfr*.



Supplementary Figure 4 Chromatograms of fluorescent peak traces showing mixed alleles (arrow heads) of codons encoding amino acid at positions 51, 59, and 108 of PfDHFR in the transfected parasites pDT-HB3 and pDT-Dd2.



Supplementary Figure 5. Chemical structures of selected DHFR inhibitors and epithelial sodium channel blockers showing similarity in structures.

Supplementary Table 1. *Plasmodium falciparum* lines, origins, assay performance metrics, and IC_{50} values (nM) of four antimalarial compounds assayed in 96- and 1536-well plate formats

Line	Origin	Plate format	S:B[1] ratio	Z' Factor	Intra-plate Controls				qHTS	
					MQ		ART		CQ[2]	QN
					Mean	SD	Mean	SD		
Dd2	From W2	96			6		9		180	120
		1536-1	13	0.77	33	5	30	3	10,000	320
		1536-2	10	0.72	45	3	33	9	10,000	400
HB3	Honduras	96			13		14		10	140
		1536-1	9	0.8	8	2	25	2	250	160
		1536-2	9	0.72	10	2	21	6	320	160
3D7	Europe	96			13		21		8	31
		1536-1	23	0.77	15	3	28	3	100	80
		1536-2	21	0.77	27	10	22	6	130	100
7G8	Brazil	96			13		9		90	110
		1536-1	13	0.72	6	2	9	1	2500	250
		1536-2	10	0.75	13	1	4	1	1600	130
GB4	Ghana	96			5		9		82	36
		1536-1	12	0.69	7	1	ND		1600	160
		1536-2	8	0.8	12	1	5	1	1600	130
D10	PNG	96			7		2		13	40
		1536-1	10	0.8	62	4	15	1	320	80
		1536-2	13	0.81	69	8	19	2	320	80
W2	Indochina	96			7		5		160	300
		1536-1	16	0.78	12	1	8	1	2000	160
		1536-2	18	0.79	13	2	11	1	2000	320

[1] S:B ratio, signal to background ratio. S:B ratio and Z' factor indicate mean values from 10–12 plates from each replicate.

[2] The discrepancy in chloroquine IC_{50} between 96- and 1536-well plate formats was due to the low solubility of chloroquine in DMSO for the qHTS (data not shown). Chloroquine was dissolved in ethanol for the 96-well assay. PNG, Papua New Guinea. ND, no data.

Supplementary Table 2. Consensus IC_{50} values and activity categories of the LOPAC compounds tested for seven *Plasmodium falciparum* lines

Supplementary Table 3. Number and percentage of compounds in different activity categories for seven *Plasmodium falciparum* strains

	3D7			7G8			D10			Dd2			GB4			HB3			W2		
	1	2	Con[1]	1	2	Con	1	2	Con	1	2	Con	1	2	Con	1	2	Con	1	2	Con
Active[2]	270	282	243	323	348	309	221	232	203	196	109	107	313	353	293	274	267	243	424	506	410
Active/Inconclusive			65			52			42			81			79			53			107
Inconclusive	293	336	244	336	342	269	258	315	215	279	237	156	391	344	284	284	314	237	370	358	255
Discrepant			0			1			0			10			1			2			2
Not Active	716	661	727	620	589	648	800	732	819	804	933	925	575	582	622	721	698	744	485	415	505
% Active[3]	21%	22%	24%	25%	27%	28%	17%	18%	19%	15%	9%	15%	24%	28%	29%	21%	21%	23%	33%	40%	40%
% Inconclusive	23%	26%	19%	26%	27%	21%	20%	25%	17%	22%	19%	12%	31%	27%	22%	22%	25%	19%	29%	28%	20%
% Not Active	56%	52%	57%	48%	46%	51%	63%	57%	64%	63%	73%	72%	45%	46%	49%	56%	55%	58%	38%	32%	39%
% Agreement[4]			100%			100%			100%			95%			100%			99%			100%

[1] Con, consensus activity between the two replicate assays.

[2] Active, inhibitors with Class 1 or 2.1 curves in both replicates; Active/Inconclusive, active in one replicate but inconclusive in the other replicate; Inconclusive, inhibitors with Class 2.2 or 3 curves; Discrepant, active in one replicate but not active in the other replicate, Not Active, compounds with Class 4 curves.

[3] % Active, percentage of Active and Active/Inconclusive compounds.

[4] % Agreement, percentage of consensus Active and Active/Inconclusive minus the Discrepant actives.

Supplementary Table 4. Potency ranges of consensus actives from each *Plasmodium falciparum* line

Potency Range	3D7	7G8	D10	Dd2	GB4	HB3	W2
< 0.1 μM	12	4	4	0	7	4	6
0.1-1 μM	32	29	17	12	32	27	59
1-10 μM	156	244	121	125	176	154	317
>10 μM	108	84	103	51	157	111	135
Total	308	361	245	188	372	296	517

Compound potencies were averaged from two replicates.

Supplementary Table 5. Potency ranges of 155 compounds active in all seven *Plasmodium falciparum* lines

Potency range	3D7	7G8	D10	Dd2	GB4	HB3	W2
< 0.1 μM	6%	3%	3%	0%	4%	2%	3%
0.1–1 μM	19%	19%	10%	7%	16%	16%	29%
1–10 μM	64%	75%	59%	66%	71%	69%	66%
> 10 μM	11%	3%	28%	26%	9%	13%	2%

Compound potencies were averaged from two replicates.

Supplementary Table 6. Highly potent compounds against all *Plasmodium falciparum* lines

Sample name	3D7	7G8	D10	Dd2	GB4	HB3	W2	Sample description	References	Human trial
Quinidine sulfate	0.03	0.04	0.02	0.16	0.03	0.04	0.06	Na^+ channel blocker		Yes
Diphenyleneiodonium chloride	0.06	0.09	0.1	1	0.07	0.06	0.08	Endothelial nitric oxide synthase inhibitor	No report	No information
Dequalinium dichloride (**8**)	0.07	0.09	0.1	0.56	0.01	0.1	0.14	Selective blocker of apamin-sensitive K+ channels	Exp Parasitol. 2007 115:19-24.	Yes
Methotrexate	0.07	0.11	0.04	0.09	0.04	0.07	0.04	Folic acid antagonist	J Med Chem. 1978 2:1059-70.	Yes
p-F-HHSiD (**7**)	0.07	0.06	0.03	0.13	0.04	0.07	0.07	High-affinity muscarinic acetylcholine receptor antagonist	No report	No information
Emetine dihydrochloride hydrate	0.09	0.1	0.14	0.13	0.09	0.11	0.12	Apoptosis inducer; RNA-protein translation inhibitor	Lancet. 1985 2(8458):784.	Yes
Pentamidine isethionate	0.09	0.26	0.19	1	0.28	0.25	0.23	K^+ channel blocker; antimalarial		Yes
Quinine sulfate (5)	0.09	0.19	0.08	0.35	0.16	0.17	0.22	Monoamine oxidase inhibitor; antimalarial		Yes
Quinacrine dihydrochloride	0.1	0.12	0.09	0.19	0.08	0.1	0.15	Antimalarial; anti-amoebic		Yes
Dequalinium analog, C-14 linker	0.15	0.3	0.32	0.35	0.32	0.33	0.13	Protein kinase C-α (PKC-α) inhibitor	Exp Parasitol. 2007 115:19-24.	No information
Ro 90-7501	0.28	0.52	0.74	1	0.74	0.83	0.22	Inhibits amyloid β42 (Aβ42) fibril formation.	No report	No information
Taxol (**9**)	0.09	0.25	0.15	0.15	1.78	0.23	0.07	Inhibitor of microtubule assembly	J Clin Invest. 1994 94:413-7.	Yes
BW 284c51 (**10**)	0.09	0.47	0.28	1.86	0.22	0.28	0.08	Selective acetylcholinesterase inhibitor	No report	No information
WB 64	0.09	0.56	0.52	1.86	0.35	0.63	0.66	M2 muscarinic acetylcholine receptor modulator	No report	No information
Tyrphostin A9	0.52	1.05	1.05	0.93	0.93	1	0.32	Selective PDGF tyrosine kinase receptor inhibitor	No report	No information
Ellipticine	0.56	0.93	1.78	1.41	0.4	1.66	0.52	CYP1A1 and DNA topoisomerase II inhibitor	Mem Inst Oswaldo Cruz. 2007 102:359-	No information
U-83836 dihydrochloride	0.59	0.4	0.63	1.58	0.42	0.74	0.42	Free radical lipid peroxidation inhibitor	No report	No information
Mitoxantrone	0.59	0.93	1.48	1	1.41	0.83	1.12	DNA synthesis inhibitor	No report	Yes
Cyclosporin A	0.63	0.56	1.26	0.71	0.56	0.83	0.47	Calcineurin phosphatase inhibitor	Agents Actions 1981 11:770-3.	Yes
Idarubicin	0.71	1.05	1.26	1.41	1.05	1.17	1	Antineoplastic	No report	Yes
(S)-(+)-Camptothecin	0.83	1.41	1.12	1.32	0.89	0.89	0.56	DNA topoisomerase I inhibitor	Biochem Pharmacol. 1998 55:709-11.	Yes
Niclosamide	0.89	0.89	1	1.48	0.74	0.89	0.56	Protonophoric anthelmintic	No report	Yes
Propafenone hydrochloride	0.93	0.35	1.78	1.05	0.33	0.93	0.23	β-adrenoceptor antagonist	Chem Biol Drug 2006, 67:409-16.	Yes
Calcimycin	1	1.05	1.32	1.41	1.32	1.32	1	Ca2+ ionophore	J Cell Biol. 93:680-4.	Yes
(S)-(-)-propafenone hydrochloride	1.05	0.3	1.78	1.26	0.32	1.05	0.28	β-adrenoceptor blocker	Chem Biol Drug Des. 2006 67:409-16.	Yes

The IC_{50} values were averaged from two replicates. Names in bold are known antimalarial drugs. p-F-HHSiD is hexahydro-sila-difenidol hydrochloride, p-fluoro analog.

Supplementary Table 7. Compounds with five-fold or greater differential potency between two or more isolates

			Log IC_{50}							Activity Category						
Sample ID	Sample Name	Delta IC50	3D7	7G8	D10	Dd2	GB4	HB3	W2	3D7	7G8	D10	Dd2	GB4	HB3	W2
NCGC00093809-01	Diphenhydramine hydrochloride	1.9	-4.5	-5.5	-4.5	-5.4	-6.5	-4.5	-6.1	N	I	N	I	A	N	A
NCGC00093999-01	4-Hydroxy-3-methoxyphenylacetic acid	1.9	-4.5	-4.5	-4.5	-4.5	-4.5	-4.5	-6.5	N	N	N	N	N	N	A*
NCGC00093746-01	Chloroquine diphosphate	1.9	-6.9	-5.7	-6.5	-5.0	-5.8	-6.5	-5.7	A	A	A	A*	A	A	A
NCGC00093720-01	(?)-Chlorpheniramine maleate	1.8		-5.4	-4.5		-6.4	-4.5	-6.0	N*	I	N	D	A	N	A
NCGC00094455-01	Vincristine sulfate	1.8	-7.0	-6.0	-6.3	-7.0	-5.5	-5.8	-7.3	A	I	A*	A	I	A	A*
NCGC00093777-01	Dequalinium dichloride	1.8	-7.2	-7.0	-7.0	-6.3	-8.1	-7.0	-6.9	A	A*	A	A	A	A	A*
NCGC00025233-02	SB 415286	1.8			-4.5	-4.5	-5.0	-6.3	-4.9	N*	I*	N	N	I	A*	I
NCGC00093676-01	(?)-Brompheniramine maleate	1.7	-4.5	-5.8	-4.5		-6.3	-4.5	-6.1	N	A*	N	D	A	N	A
NCGC00094204-01	Orphenadrine hydrochloride	1.6	-5.0	-5.4	-4.5	-5.9	-5.6		-6.2	I	I	N	A	I	I*	A
NCGC00093697-01	(+)-Brompheniramine maleate	1.6		-5.8	-4.5	-5.2	-6.1	-4.5	-6.1	N*	A*	N	I	A	N	A
NCGC00094216-01	Nisoxetine hydrochloride	1.6	-4.9	-5.6	-4.5	-5.6		-4.5	-6.1	I	I	N	A	I*	N	A
NCGC00093776-01	Dextromethorphan hydrobromide monohydrate	1.5		-5.6	-4.5		-5.2	-4.9	-6.1	N*	I	N	D	I	I	A
NCGC00093722-01	(+)-Chlorpheniramine maleate	1.5	-4.5	-5.3	-4.5	-5.2	-6.0	-4.5	-5.9	N	I	N	I	A*	N	A
NCGC00094111-01	R(-)-Me5	1.5	-4.5	-5.2	-4.5	-5.2	-6.0	-4.5	-5.9	N	I	N	I	A	N	A
NCGC00023458-04	Triamterene	1.5	-5.7	-4.9	-5.6	-4.5	-6.0	-5.0	-4.9	A	I	A	N	A	I	I
NCGC00094186-01	Memantine hydrochloride	1.5		-5.0	-4.5	-5.1	-6.0		-5.8	N*	I	N	I	A	N*	A
NCGC00093683-01	Benzamil hydrochloride	1.4	-6.3	-5.7	-6.1	-4.9	-5.5	-6.0	-5.4	A	A	A	I	A	A	A
NCGC00093814-01	Decamethonium dibromide	1.4	-6.4	-5.3	-6.1	-5.0	-5.5	-5.8	-6.0	A	A	A	A*	A	A	A
NCGC00024995-02	Taxol	1.4	-7.1	-6.6	-6.8	-6.8	-5.8	-6.6	-7.2	A	A	A	A	A	A	A
NCGC00093618-01	Alaproclate hydrochloride	1.4		-5.1	-4.5	-5.1	-5.9		-5.9	N*	I	N	I	A*	N*	A
NCGC00093610-01	BW 284c51	1.4	-7.0	-6.3	-6.6	-5.7	-6.7	-6.6	-7.1	A	A	A	A	A	A	A

NCGC00024707-04	Trimethoprim	1.4	-5.9	-4.5	-5.5	-4.5	-5.9	-5.0		A	N	A	N	A	A*	N*
NCGC00094476-01	R(+)-Terguride	1.4	-6.6	-5.4	-5.6	-5.2	-5.2	-5.9	-6.2	A	A*	I	I	A*	A*	A
NCGC00093863-01	Dextrorphan D-tartrate	1.3	-4.5	-5.2	-4.5	-5.2	-5.9	-4.5	-5.8	N	I	N	I	A	N	A
NCGC00094089-01	Mibefradil dihydrochloride	1.3	-5.5	-5.7	-5.1	-6.0	-6.4	-5.3	-6.4	A	A	A	A	A	A	A
NCGC00094505-01	WB 64	1.3	-7.0	-6.3	-6.3	-5.7	-6.5	-6.2	-6.2	A	A	A	A	A	A	A
NCGC00092339-02	N-(3,3-Diphenylpropyl)glycinamide	1.3		-5.4	-4.5	-5.2	-5.8	-4.5	-5.6	N*	I	N	I	A	N	A
NCGC00093760-01	Carbetapentane citrate	1.3	-4.9	-5.4		-5.5	-6.2	-5.0	-6.2	I	I	N*	I	A*	I	A
NCGC00093793-01	Dihydroergotamine methanesulfonate	1.3	-5.7	-6.7	-5.4	-5.5	-5.7	-6.1	-5.9	A	A	A	A	A	A	A
NCGC00093801-01	Diphenyleneiodonium chloride	1.3	-7.2	-7.1	-7.0	-6.0	-7.2	-7.3	-7.1	A	A	A	A	A	A	A
NCGC00094262-01	Promazine hydrochloride	1.2	-4.9	-5.1	-4.8	-5.8	-5.1	-5.0	-6.0	I	A*	I	A	A*	I	A
NCGC00094461-01	U-74389G maleate	1.2	-6.2	-6.0	-5.9	-5.0	-5.9	-5.9	-6.1	A	A	A	A	A	A	A
NCGC00094449-01	Tomoxetine	1.2	-4.7	-5.2	-4.5	-5.3	-5.8	-4.5		I	I	N	I	I	N	I*
NCGC00094189-01	3-Methoxy-morphanin hydrochloride	1.2	-5.0	-5.4	-4.9		-5.2	-4.9	-6.1	I	A	I	D	I	I	A
NCGC00094049-01	3-(1H-Imidazol-4-yl)propyl di(p-fluorophenyl)methyl ether hydrochloride	1.2	-5.9	-5.7	-5.4	-5.0	-6.0	-5.7	-6.2	A	A	A	A	A	A	A
NCGC00093599-01	SKF 97541 hydrochloride	1.2	-5.4	-5.5	-5.7	-4.5	-5.5	-5.6	-5.6	I	I	A*	N	I	I	A
NCGC00094451-01	Telenzepine dihydrochloride	1.2	-5.5		-5.4	-4.5	-5.7	-5.4		I	N*	I	N	I	I	I*
NCGC00093914-01	Guanfacine hydrochloride	1.2	-5.5	-5.3	-5.3	-4.5	-5.4	-5.7	-5.6	A	A*	I	N	A*	A*	A
NCGC00093681-01	ML-9	1.2	-4.9	-5.2	-4.7	-5.2	-5.4		-5.9	A*	A*	I	I	I	N*	A
NCGC00094057-01	Ketotifen fumarate	1.2	-5.5	-5.0	-5.4		-5.5	-5.0	-6.1	A	I	A	D	A	I	A
NCGC00094469-01	Vinblastine sulfate salt	1.1	-6.4	-6.0	-5.3	-6.4	-5.6	-5.6	-6.4	A	A	A*	A	A	A	A
NCGC00094196-01	(+)-MK-801 hydrogen maleate	1.1	-4.5	-5.4	-4.5	-5.1	-5.6	-4.5	-5.7	N	I	N	I	I	N	A
NCGC00094457-01	Yohimbine hydrochloride	1.1	-5.2	-5.7	-5.4	-4.5	-5.5	-5.4	-5.7	I	I	I	N	I	I	A*
NCGC00094070-01	L-750,667 trihydrochloride	1.1	-5.0	-5.1	-4.8	-4.5	-4.9	-5.0	-5.6	I	I	I	N	I	A*	A
NCGC00093543-01	Sodium Taurocholate	1.1	-5.2	-4.5		-4.5	-5.4	-5.6	-5.5	A*	N	N*	N	I	A	A

NCGC00093842-01	6,7-Dichloroquinoxaline-2,3-dione	1.1	-5.6			-4.5	-5.4	-5.6	-5.5	A	N*	N*	N	I	A	A
NCGC00093759-01	CNS-1102	1.1	-4.9	-5.5	-4.9		-4.9	-5.0	-6.0	A	A	A	D	A	A	A
NCGC00093732-01	4-Chloromercuribenzoic acid	1.1	-5.6	-5.7	-5.8	-4.9	-5.5	-5.3	-6.0	A	A	A	A*	A	A	A
NCGC00094488-01	VUF 5574	1.1	-4.5	-5.6	-4.5	-4.5	-4.5	-4.5		N	I	N	N	N	N	N*
NCGC00094054-01	Imipramine hydrochloride	1.1	-4.9	-5.3	-4.8		-5.1	-4.9	-5.9	I	A	I	D	A*	I	A
NCGC00093831-01	Doxycycline hydrochloride	1.1	-5.9	-5.4	-5.0	-5.3	-5.2	-5.3	-6.1	A	A	A*	A	A	A	A
NCGC00094222-01	Nylidrin hydrochloride	1.0	-4.9	-5.1	-4.5	-5.2	-5.5		-5.6	I	A*	N	I	A*	N*	A
NCGC00094236-01	Pentamidine isethionate	1.0	-7.0	-6.6	-6.7	-6.0	-6.6	-6.6	-6.6	A	A	A	A	A	A	A
NCGC00094179-01	Methapyrilene hydrochloride	1.0	-4.5	-5.2	-4.5	-5.1	-5.3	-4.5	-5.6	N	I	N	I	A	N	A
NCGC00093798-01	CGP 20712A methanesulfonate	1.0	-5.6	-5.0	-5.5	-4.5	-4.9	-5.1		A	A*	A	N	I	A	I*
NCGC00093782-01	4'-Chloro-3-alpha-(diphenylmethoxy)tropane hydrochloride	1.0	-5.6	-5.6	-5.2	-5.1	-5.6	-5.6	-6.1	A	A	A	A	A	A	A
NCGC00094135-01	Minocycline hydrochloride	1.0	-6.1	-5.4	-5.1	-5.4	-5.3	-5.3	-6.1	A	A	A	A*	A	A	A
NCGC00094021-01	VER-3323 hemifumarate salt	1.0	-5.0	-5.0		-5.0	-5.9	-4.9	-5.7	I	A	I*	I	A	I	A
NCGC00094264-01	1,3-PBIT dihydrobromide	1.0	-5.5	-5.1	-5.2	-5.1	-5.6	-5.1	-6.1	A	A	A	A*	A	A	A
NCGC00091250-02	Reserpine	1.0	-5.7	-6.5	-5.6	-5.6	-5.7	-5.9	-6.1	A	A	A	A	A	A	A
NCGC00092329-02	Eliprodil	1.0	-5.8	-6.4	-5.6	-5.8	-6.5	-5.9	-6.6	A	A	A	A	A	A	A
NCGC00093840-01	JHW 007 hydrochloride	1.0	-6.1	-5.8	-5.5	-5.4	-5.9	-6.0	-6.4	A	A	A	A	A	A	A
NCGC00093797-01	(+)-Cyclazocine	1.0	-4.5	-5.1	-4.5	-5.1	-4.9	-4.5	-5.5	N	I	N	I	A	N	A
NCGC00094255-01	Bisoprolol hemifumarate salt	1.0	-4.5		-4.5	-4.5		-4.5	-5.5	N	N*	N	N	N*	N	A*
NCGC00094281-01	Pheniramine maleate	1.0	-4.5	-5.3	-4.5	-5.0	-5.3	-4.5	-5.5	N	I	N	I	I	N	A*
NCGC00094297-01	Picotamide	1.0	-4.5	-5.5	-4.5	-4.5	-4.5	-4.5		N	A*	N	N	N	N	N*
NCGC00093949-01	17alpha-hydroxyprogesterone	1.0	-5.0		-4.5	-4.5			-5.5	I	N*	N	N	I*	N*	A
NCGC00093779-01	DM 235	1.0	-5.4		-5.5	-4.5	-5.5	-5.5	-5.4	I	N*	I	N	I	I	I
NCGC00024840-02	Palmitoylethanolamide	1.0			-4.5	-4.5	-5.5	-5.0	-5.4	N*	N*	N	N	A*	I	A*

NCGC00093941-01	Fluphenazine dihydrochloride	1.0	-5.6	-5.5	-5.2	-5.0	-5.5	-5.4	-5.9	A	A	A	A*	A	A	A
NCGC00093556-01	Amperozide hydrochloride	1.0	-6.0	-5.9	-5.7	-5.6	-6.0	-6.1	-6.5	A	A	A	A	A	A	A
NCGC00093915-01	Efaroxan hydrochloride	0.9	-4.5	-5.2	-4.5	-5.0	-4.9	-4.5	-5.5	N	I	N	I	A	N	A
NCGC00094423-01	Tetraethylthiuram disulfide	0.9	-5.5	-5.2	-4.5		-5.4	-5.5	-5.0	A*	I	N	N*	I	I	I
NCGC00093686-01	Bromoacetyl alprenolol menthane	0.9	-5.5	-5.5	-5.2	-5.5	-6.1	-5.4	-6.1	A	A	A	A	A	A	A
NCGC00094302-01	Quinidine sulfate	0.9	-7.6	-7.4	-7.7	-6.8	-7.6	-7.4	-7.2	A	A	A	A	A	A	A
NCGC00093780-01	2-Cyclooctyl-2-hydroxyethylamine hydrochloride	0.9	-4.5	-5.0	-4.5	-5.0	-5.3	-4.5	-5.5	N	I	N	I	A*	N	A
NCGC00093886-01	(R)-(-)-DOI hydrochloride	0.9	-4.5	-5.1	-4.5	-5.0	-5.0	-4.5	-5.5	N	I	N	I	A*	N	A
NCGC00094200-01	NAN-190 hydrobromide	0.9	-4.9	-4.5	-4.5	-4.5		-5.2	-5.5	I	N	N	N	N*	I	A*
NCGC00093846-01	3-deazaadenosine	0.9	-5.3	-4.5	-5.5	-4.5	-5.3			I	N	A*	N	A*	D	I*
NCGC00094293-01	Phenamil methanesulfonate	0.9	-5.5	-5.0	-5.1	-4.5	-4.9	-5.1	-5.2	A	A	A	N	A	A	A
NCGC00094320-01	(?)-threo-1-Phenyl-2-decanoylamino-3-morpholino-1-propanol hydrochloride	0.9	-5.3	-5.9	-5.0	-5.6	-5.8	-5.2	-5.9	A	A	A	A	A	A	A
NCGC00094482-01	T-1032	0.9	-5.4	-6.1	-5.2	-5.6	-5.6	-5.6	-5.6	A	A	A	A	A	A	A
NCGC00093664-02	(+)-Butaclamol hydrochloride	0.9	-5.5	-5.3	-5.1	-4.8	-5.3	-5.3	-5.7	A	A	A	I	A	A	A
NCGC00093884-01	Doxazosin mesylate	0.9	-5.5	-5.7	-5.6	-5.0	-5.5	-5.6	-5.9	A	A	A	A	A	A	A
NCGC00093921-01	cis-(Z)-Flupenthixol dihydrochloride	0.9	-5.6	-5.6	-5.1	-5.0	-5.7	-5.4	-5.9	A	A	A	A*	A	A	A
NCGC00093761-01	Clotrimazole	0.9	-6.2	-6.6	-6.1	-5.7	-6.5	-6.5	-6.5	A	A	A	A	A	A	A
NCGC00094234-01	Oxybutynin Chloride	0.9	-5.2	-5.3	-5.0	-4.9	-5.3	-5.2	-5.8	A	A	A	I	A	A	A
NCGC00094395-01	Tyrphostin AG 528	0.9	-5.5	-5.3	-5.5	-4.8	-5.1	-5.6	-5.4	A	I	A	I	I	A	A
NCGC00094230-01	Propafenone hydrochloride	0.9	-6.0	-6.5	-5.8	-6.0	-6.5	-6.0	-6.6	A	A	A	A	A	A	A
NCGC00025304-02	Indirubin-3'-oxime	0.9	-6.5	-6.0	-5.9	-5.7	-6.3	-6.3	-6.5	A	A	A	A	A	A	A
NCGC00093879-01	(?)-DOI hydrochloride	0.9	-4.5	-5.0	-4.5	-5.0	-5.2	-4.5	-5.4	N	A*	N	I	A*	N	A
NCGC00094359-01	Ro 25-6981 hydrochloride	0.9	-4.8	-5.1	-4.9	-4.9	-4.9	-4.5	-5.4	I	A*	I	I	A*	N	A
NCGC00093785-01	Doxylamine succinate	0.9	-4.9			-5.0	-5.3	-4.5	-5.4	I	N*	N*	I	A*	N	A

NCGC00094407-01	BRL 52537 hydrochloride	0.9	-4.9	-5.3		-5.0	-5.8		-5.7	I	A*	I*	I	A*	I*	A
NCGC00093794-01	Desipramine hydrochloride	0.9	-5.0	-5.3	-5.0	-5.7	-5.0	-4.9	-5.8	A	A	I	A*	A*	I	A
NCGC00093783-01	Droperidol	0.9	-5.5	-5.3	-5.8	-5.0	-5.2	-5.6	-5.5	A	A	A	I	I	A*	A
NCGC00094456-01	WIN 62,577	0.9	-5.6	-6.2	-5.4	-5.5	-5.4	-5.3	-5.6	A	A	A*	A	A	A	A
NCGC00094479-01	(?)-Verapamil hydrochloride	0.9	-5.8	-5.2	-5.3	-5.4	-5.3	-5.6	-6.0	A	A	I	I	A*	A*	A
NCGC00094487-01	R(+)-UH-301 hydrochloride	0.9	-5.8	-6.1	-5.6	-5.7	-6.5	-5.9	-6.2	A	A	A	A	A	A	A
NCGC00025380-02	Dihydroergocristine methanesulfonate	0.9	-6.1	-6.5	-5.7	-5.8	-6.0	-6.1	-6.2	A	A	A	A	A	A	A
NCGC00093837-01	3',4'-Dichlorobenzamil	0.9	-6.5	-6.4	-6.4	-5.7	-6.1	-6.3	-6.1	A	A	A	A	A	A	A
NCGC00093699-01	Phenoxybenzamine hydrochloride	0.9		-5.1	-5.0		-5.8	-4.9	-5.6	N*	I	I	N*	I	I	A
NCGC00094136-01	(-)-MK-801 hydrogen maleate	0.8	-4.5		-4.5	-5.0	-5.4	-4.5	-5.4	N	N*	N	I	I	N	A
NCGC00094064-01	ICI 118,551 hydrochloride	0.8	-5.0	-5.6	-4.9	-5.0	-5.6	-5.0	-5.7	A*	A	I	I	A	I	A
NCGC00093778-01	Doxepin hydrochloride	0.8	-5.3	-5.3	-5.1	-5.8	-5.5	-5.1	-5.9	A*	I	I	A	I	I	A
NCGC00094101-01	L-703,606 oxalate	0.8	-5.3	-5.1	-5.0	-5.4	-5.3	-5.0	-5.8	A	A	A	A	A	A	A
NCGC00094022-01	NNC 55-0396	0.8	-5.5	-5.6	-5.3		-6.0	-5.4	-6.1	A	A	A	A	A	A	A
NCGC00094300-01	(?)-PPHT hydrochloride	0.8	-5.5	-6.1	-5.3	-5.6	-6.1	-5.6	-6.1	A	A	A	A	A	A	A
NCGC00025130-02	SB 228357	0.8	-5.8	-5.8	-5.7	-5.1	-5.8	-6.0	-5.6	A	A	A	A	A	A	A
NCGC00093960-01	GR 125487 sulfamate salt	0.8	-5.2	-5.0	-5.2	-4.5	-5.1	-5.4	-5.0	A	I	A	N	I	A	A
NCGC00025198-02	CGP-7930	0.8	-5.4	-5.1		-4.5	-4.8	-5.2	-5.1	I	I	I*	N	I	I	A*
NCGC00093642-01	Alprenolol hydrochloride	0.8	-5.0	-5.5	-5.0	-5.0	-5.7	-4.9	-5.7	A*	A	I	A	A	A	A
NCGC00094315-01	(S)-(-)-propafenone hydrochloride	0.8	-6.0	-6.5	-5.8	-5.9	-6.5	-6.0	-6.6	A	A	A	A	A	A	A
NCGC00093531-01	Amantadine hydrochloride	0.8	-4.5	-5.3	-4.5		-5.0	-4.5		N	I	N	N*	A	N	I*
NCGC00093717-01	Citalopram hydrobromide	0.8	-5.0	-5.2			-5.8		-5.7	I	I	N*	N*	A*	N*	A
NCGC00094314-01	(S)-Propranolol hydrochloride	0.8	-5.0	-5.4	-4.9	-5.0	-5.5	-5.1	-5.7	A	A	I	A*	A	I	A
NCGC00094080-01	(-)-cis-(1S,2R)-U-50488 tartrate	0.8	-5.0	-5.2	-4.9	-5.1	-5.6	-4.9	-5.7	A*	I	I	I	I	I	A

NCGC00021472-02	Domperidone	0.8	-5.4	-5.8	-5.0	-5.0	-5.0	-5.1	-5.4	A	A	A	A	A	A	A
NCGC00089747-02	Budesonide	0.8	-5.5	-5.8	-5.0	-5.0	-5.5	-5.4	-5.6	A	A	A	A*	A	A	A
NCGC00094398-01	SR 59230A oxalate	0.8	-5.7	-6.3	-5.6	-5.7	-6.1	-5.8	-6.4	A	A	A	A	A	A	A
NCGC00093557-01	Atropine sulfate	0.8	-4.5		-4.5		-5.0	-4.5	-5.3	N	N*	N	N*	I	N	I
NCGC00093781-01	1-(m-Chlorophenyl)-biguanide hydrochloride	0.8	-5.0	-5.0		-4.5		-5.1	-5.3	I	I	N*	N	I*	I	A*
NCGC00024702-02	3-Tropanyl-3,5-dichlorobenzoate	0.8		-5.3		-4.5		-4.9	-5.1	I*	I	N*	N	I*	I	A*
NCGC00094212-01	(?)-Propranolol hydrochloride	0.8	-5.0	-5.4	-4.9	-4.9	-5.5	-4.9	-5.6	I	A	I	A*	A	A*	A
NCGC00094192-01	Nortriptyline hydrochloride	0.8	-5.0	-5.1	-4.9	-5.4	-5.2	-4.9	-5.6	A*	A	I	A*	A*	I	A
NCGC00024221-03	AA-861	0.8	-5.1	-5.7	-4.9	-5.0	-5.5	-5.0	-5.4	A	A	A	A*	A	A	A
NCGC00023875-04	Haloperidol	0.8	-5.5	-5.3	-5.0	-5.3	-5.5	-5.1	-5.7	A	A	A	A	A	A	A
NCGC00093763-01	Cantharidic Acid	0.8	-5.7	-5.4	-5.7	-5.1	-5.5	-5.6	-5.9	A	A	A	A	A	A	A
NCGC00094112-01	BIO	0.8	-5.7	-5.5	-5.4	-5.0	-5.7	-5.7	-5.8	A	A	A	A	A	A	A
NCGC00094187-01	Methoctramine tetrahydrochloride	0.8	-6.0	-5.8	-5.8	-5.4	-5.7	-5.7	-6.1	A	A	A	A	A	A	A
NCGC00094104-01	MG 624	0.8	-6.1	-5.9	-6.3	-5.7	-6.0	-6.1	-6.4	A	A	A	A	A	A	A
NCGC00093981-01	(+)-Hydrastine	0.7	-4.5	-4.5	-4.5	-4.5		-4.5	-5.3	N	N	N	N	I*	N	A*
NCGC00093838-01	3,4-Dihydroxyphenylacetic acid	0.7	-4.5		-4.5	-4.5	-4.5	-4.5	-5.3	N	N*	N	N	N	N	I
NCGC00093604-01	(?)-N-Allylnormetazocine hydrochloride	0.7	-5.0	-5.1	-4.5	-5.0	-4.9	-4.5	-5.3	I	I	N	I	I	N	A*
NCGC00093568-01	Aurintricarboxylic acid	0.7	-5.1	-5.0	-5.0	-4.5	-5.1	-5.3		I	I	I	N	I	I	I*
NCGC00094393-01	Trihexyphenidyl hydrochloride	0.7	-4.9	-5.3	-4.9	-5.2	-5.0	-5.0	-5.6	A*	A	A	I	A	A	A
NCGC00094215-01	Promethazine hydrochloride	0.7	-4.9	-5.2	-4.9	-5.2	-5.2	-5.0	-5.6	A	A	I	A*	A*	A*	A
NCGC00023125-04	Loratadine	0.7	-5.0	-5.7	-5.0	-5.0	-5.0	-5.2	-5.3	A	A	A	A*	A	A	A
NCGC00093935-01	Flupirtine maleate	0.7	-5.5	-5.6	-5.2	-5.0	-5.6	-5.6	-5.7	A	A	A	A*	A	A	A
NCGC00094459-01	T-0156	0.7	-5.7	-5.4	-5.4	-4.9	-5.3	-5.5	-5.6	A	A	I	I	A	A	A
NCGC00093649-01	BP 897	0.7	-5.7	-5.2	-5.2	-5.0	-5.0	-5.4	-5.6	A	A	I	I	I	I	A

NCGC00094478-01	S(-)-UH-301 hydrochloride	0.7	-5.8	-6.2	-5.6	-5.8	-6.4	-6.0	-6.3	A	A	A	A	A	A	A
NCGC00094471-01	WAY-100635 maleate	0.7		-5.7			-5.2	-5.0	-5.1	I*	A	N*	N*	I	I	A*
NCGC00094343-01	Ranolazine dihydrochloride	0.7	-4.5	-5.3	-4.5	-4.5		-4.5		N	I	N	N	N*	N	I*
NCGC00093968-01	IEM-1460	0.7	-5.0		-4.5	-4.5	-5.2	-4.5	-5.3	I	I*	N	N	A*	N	A
NCGC00093947-01	GYKI 52895	0.7		-4.9	-4.5		-5.0		-5.3	N*	I	N	N*	I	N*	A
NCGC00093597-01	Paroxetine hydrochloride hemihydrate (MW = 374.83)	0.7	-5.2	-5.2	-5.0	-4.9	-5.2	-5.2	-5.6	A	A	A	A*	A	A	A
NCGC00025182-02	N-Oleoylethanolamine	0.7	-5.2	-5.3	-5.2	-5.0	-5.5	-5.4	-5.7	A	A	A	A*	A	A	A
NCGC00093664-01	(?)-Butaclamol hydrochloride	0.7	-5.3	-5.2	-5.0	-5.1	-5.1	-5.0	-5.7	A	A	A	A	A	A	A

[1]Activity Category: A- active in both replicates; A*-active in one replicate and inconclusive in the other; I- inconclusive active and *P* value <0.05 in both replicates; I*- inconclusive active in both replicates and *P* value >0.05 in one or both replicates; N- inactive in both replicates; N*- inactive in one replicate and inconclusive in the other; D- inactive in one replicate and active in the other. For A, A*, and I, LogIC50 values are used or averaged for curve fit P values <0.05, for N, LogIC50 is set to -4.5, the highest tested concentration, and for I*, N*, and D, LogIC50 is not shown.

Supplementary Table 8. Candidate genes in the chromosome 5 locus linked to response to dihydroergotamine methanesulfonate (DHMS)

Gene	Location	Product description
PFE1200w	MAL5: 1,004,232 – 1,008,103	Hypothetical protein, conserved
PFE1205c	MAL5: 1,008,631 – 1,009,399 (-)	Hypothetical protein, conserved
PFE1210c	MAL5: 1,010,946 – 1,012,166 (-)	Hypothetical protein, conserved
PFE1215c	MAL5: 1,013,448 – 1,014,545 (-)	Developmentally regulated GTP-binding protein 1
PFE1220w	MAL5: 1,016,193 – 1,016,564	Hypothetical protein, conserved
PFE1225w	MAL5: 1,017,804 – 1,018,577	50S ribosomal subunit protein L12, putative
PFE1070c	MAL5: 870,181 – 874,567 (-)	Hypothetical protein, conserved
PFE1075c	MAL5: 875,870 – 877,558 (-)	Hypothetical protein, conserved
PFE1080w	MAL5: 878,065 – 879,759 (+)	Ribosomal large subunit pseudouridylate synthase
PFE1082c	MAL5: 880,062 – 880,352 (-)	Conserved Plasmodium protein, unknown function
PFE1085w	MAL5: 882,373 – 884,898 (+)	DEAD-box subfamily ATP-dependent helicase
PFE1090w	MAL5: 885,711 – 888,229 (+)	Nuceotide binding protein, putative
PFE1095w	MAL5: 888,654 – 894,724 (+)	Hypothetical protein, conserved
PFE1100w	MAL5: 896,013 – 896,935 (+)	Hypothetical protein, conserved
PFE1105c	MAL5: 897,460 – 899,751 (-)	Hypothetical protein, conserved
PFE1110w	MAL5: 901,409 – 902,168 (+)	Hypothetical protein, conserved
PFE1115c	MAL5: 902,608 – 904,839 (-)	s-adenosylmethionine-dependent methyltransferase
PFE1120w	MAL5: 907,832 – 936,310 (+)	Hypothetical protein, conserved
PFE1125w	MAL5: 937,310 – 938,032 (+)	50S ribosomal subunit protein L17, putative
PFE1130w	MAL5: 940,244 – 941,695 (+)	Hypothetical protein, conserved
PFE1135w	MAL5: 942,762 – 943,868 (+)	Hypothetical protein, conserved
PFE1140c	MAL5: 944,179 – 944,926 (-)	G10 protein, putative
PFE1145w	MAL5: 948,946 – 953,604 (+)	Hypothetical protein, conserved
PFE1150w	MAL5: 957,885 – 962,144 (+)	**Multidrug resistance protein (PfPgh-1)**
PFE1155c	MAL5: 963,222 – 965,039 (-)	Mitochondrial processing peptidase α subunit
PFE1160w	MAL5: 966,118 – 969,732 (+)	Hypothetical protein, conserved
PFE1165c	MAL5: 970,261 – 970,957 (-)	Hypothetical protein, conserved
PFE1170w	MAL5: 973,513 – 975,659 (+)	Hypothetical protein
PFE1173c	MAL5: 976,685 – 977,810 (-)	Outer arm dynein lc3, putative
PFE1175w	MAL5: 978,660 – 979,865 (+)	Hypothetical protein, conserved
PFE1180c	MAL5: 980,749 – 985,349 (-)	Hypothetical protein, conserved
PFE1185w	MAL5: 990,000 – 992,054 (+)	Transporter, putative
PFE1190c	MAL5: 993,428 – 993,865 (-)	Hypothetical protein, conserved
PFE1195w	MAL5: 998,748 – 1,002,119 (+)	Karyopherin β

Supplementary Table 9. Candidate genes in the chromosome 4 locus linked to response to trimethoprim (TMP) and triamterene (TAT)

Gene	Location	Product Description
PFD0815c	MAL4: 739,598 – 746,854 (-)	Hypothetical protein, conserved
PFD0820w	MAL4: 749,130 – 750,072 (+)	Hypothetical protein, conserved
PFD0825c	MAL4: 750,592 – 752,618 (-)	RNA-binding protein of pumilio/mpt5 family, putative
PFD0830w	MAL4: 755,069 – 756,895 (+)	**Dihydrofolate reductase-thymidylate synthase (DHFR)**
PFD0835c	MAL4: 757,841 - 760,693 (-)	LETM1-like protein, putative
PFD0840w	MAL4: 763,275 - 783,584 (+)	Hypothetical protein, conserved
PFD0850c	MAL4: 784,181 - 786,405 (-)	Hypothetical protein, conserved
PFD0855c	MAL4: 787,231 - 790,227 (-)	Hypothetical protein, conserved
PFD0860w	MAL4: 790,602 - 793,411 (+)	Hypothetical protein, conserved
PFD0865c	MAL4: 794,224 - 796,323 (-)	cdc2-related protein kinase 1

Supplementary Table 10. PfPgh-1 haplotypes and DHMS IC_{50} values in field isolates and transfected parasites

		PfPgh-1 haplotype					
Parasite	Origin	86	184	1034	1042	1246	IC_{50}(nM)
GB4	Africa	Y	F	S	N	D	2817 ± 134
W2	Indochina	Y	Y	S	N	D	1336 ± 153
Dd2	Indochina	Y	Y	S	N	D	3915 ± 213
D10	PNG	N	Y	S	N	D	2255 ± 171
3D7	Europe	N	Y	S	N	D	2254 ± 179
C2A	Thailand	N	F	S	N	D	3211 ± 240
224	Africa	N	F	S	N	D	3151 ± 191
HB3	Honduras	N	F	S	D	D	891 ± 73
ECU	Ecuador	N	F	S	D	D	1035 ± 159
JCK	Colombia	N	F	S	D	D	895 ± 19
7G8	Brazil	N	F	C	D	Y	371 ± 10
PC15	Peru	N	F	C	D	D	410 ± 12
PC26	Peru	N	F	C	D	D	297 ± 4
PC49	Peru	N	F	C	D	D	293 ± 8
DIV14	Brazil	N	F	C	D	Y	308 ± 10
PAD	Brazil	N	F	C	D	Y	267 ± 24
DIV17	Brazil	N	F	C	D	Y	255 ± 30
DIV30	Brazil	N	F	C	D	Y	184 ± 18
$7G8^{D10}$	MRA-566	N	F	S	N	D	3586 ± 242
$D10^{D10}$	MRA-563	N	Y	S	N	D	3901 ± 80
$D10^{7G8/2}$	MRA-565	N	Y	S(C)	N(D)	D(Y)	2457 ± 263
$D10^{7G8/1}$	MRA-564	N	Y	C(S)	D(N)	Y	276 ± 29
$7G8^{7G8}$	MRA-567	N	F	C	D	Y	333 ± 34
SND^{GCO3}	Sidhu *et al.*	N	F	S	N	D	2713 ± 145
SND^{3BA6}	Sidhu *et al.*	N	F	S	N	D	2122 ± 32
GCO3	Sidhu *et al.*	N	F	S	D	D	1089 ± 127
SDD^{GCO3}	Sidhu *et al.*	N	F	S	D	D	874 ± 144
3BA6	Sidhu *et al.*	N	F	S	D	D	864 ± 126
SDD^{3BA6}	Sidhu *et al.*	N	F	S	D	D	914 ± 15
CDY^{GCO3}	Sidhu *et al.*	N	F	C	D	Y	42 ± 33
CDY^{3BA6}	Sidhu *et al*.	N	F	C	D	Y	46 ± 10

Parasites of origin MRA-563 to MRA-567 (from MR4, ATCC) were allelic exchanged clones initially described in Reed *et al*. (Nature 403, 906–909). Alleles in parenthesis were expected from the published data but could not be confirmed after sequencing DNA samples from the parasites. Parasites from Sidhu *et al*. [19] were also engineered as *pfmdr1* modified clones. DMHS is dihydroergotamine methanesulfonate. Origins of the parasites were either the geographic origins or sources where they were obtained.

Supplementary Table 11. Effects of verapamil on parasite response to DHMS

Parasite	Origin	PfPgh-1 polymorphism					IC_{50} w/o ver (nM ± SD)	IC_{50} with ver (nM ± SD)
		86	184	1034	1042	1246		
GCO3	Sidhu *et al*.	N	F	S	D	D	1089 ± 127	1058 ± 218
SDD^{GCO3}	Sidhu *et al*.	N	F	S	D	D	874 ± 144	933 ± 195
SND^{GCO3}	Sidhu *et al*.	N	F	S	N	D	2713 ± 145	2810 ± 231
CDY^{GCO3}	Sidhu *et al*.	N	F	C	D	Y	42 ± 33	71 ± 22
3BA6	Sidhu *et al*.	N	F	S	D	D	864 ± 124	1010 ± 17
SDD^{3BA6}	Sidhu *et al*.	N	F	S	D	D	914 ± 15	1256 ± 103
SND^{3BA6}	Sidhu *et al*.	N	F	S	N	D	2122 ± 32	2095 ± 211
CDY^{3BA6}	Sidhu *et al*.	N	F	C	D	Y	46 ± 10	22 ± 8

Parasite responses (IC_{50}) to DHMS were obtained in the absence and presence of 0.8 μM of verapamil.

Supplementary Table 12. PfDHFR alleles and IC_{50} to trimethoprim and triamterene from parasite lines and transfected parasites

		PfDHFR polymorphism				
Parasite	Origin	51	59	108	μM TMP IC_{50}	μM TAT IC_{50}
3D7	Europe	N	C	S	2.6 ± 0.3	4 ± 1
GB4	Africa	N	C	S	2.8 ± 0.1	2.2 ± 0.4
D10	PNG	N	C	S	4.1 ± 0.4	5.3 ± 0.7
HB3	Honduras	N	C	T	30 ± 4	70 ± 2
7G8	Brazil	I	C	T	158 ± 0.01	176 ± 1
Dd2	Indochina	I	R	T	194 ± 13	154 ± 4
W2	Indochina	I	R	T	169 ± 2	141 ± 2
pDT-HB3	Transgenic	N	C	S/T	40 ± 0.7	95 ± 1
pDT-Dd2	Transgenic	N/I	C/R	S/T	117 ± 24	95 ± 1

Note: Higher IC_{50} values for parasites with mutant alleles (compared with those in **Fig. 3** and **Supplementary Fig. 2**) were caused by reduced solubility after increasing initial drug concentration from 125 μM to 400 μM to obtain a more complete curve. The changes in IC_{50}s did not change our conclusion that mutations in *pfdhfr* confer resistance to TMP and TAT.

Supplementary Methods:

Supplementary Table 13. qHTS protocol for the malaria SYBR green assay.

Step	Parameter	Value	Description
1	Reagent	3 uL	Growth medium
2a	Library Compounds	23 nL	29 uM to 0.4 nM titration series
2b	Control compounds	23 nL	0.29 uM
3	Reagent	5 uL	Malaria-infected RBCs
4	Time	72 hr	37 C incubation
5	Reagent	2 uL	Lysis buffer + SYBR Green
6	Time	Overnight	Ambient incubation
7	Detector	Fluorescence	EnVision

Notes	
1	Assay adopted from Plouffe et al 2008 *PNAS* 105: 9059. Reagents were dispensed into1536-well black clear plate (Aurora Biotechnologies) using a Multidrop Combi (Thermo Fisher Scientific Inc.) contained in a biosafety cabinet.
2	LOPAC1280 collection (SigmaAldrich) at eight 5-fold dilutions beginning at 10 mM, 348-fold dilution in assay volume following pin transfer
3	0.29 uM artemisinin and 0.58 uM mefloquine
4	0.3% parasitemia, 2.5% hematocrit
5	5% CO_2, 100% humidity
6	Lysis buffer: 20 mM Tris-HCl, 10 mM EDTA, 0.16% Saponin, 1.6% Triton-X, 10X SYBR Green. 25 sec shake immediately following dispense.
7	Approximately 18 hours
8	EnVision (PerkinElmer) bottom read at 485/14nm excitation and 535/25 nm emission