Supplementary material

Corrigendum: A single amino acid residue can determine sensitivity of SERCAs to artemisinins

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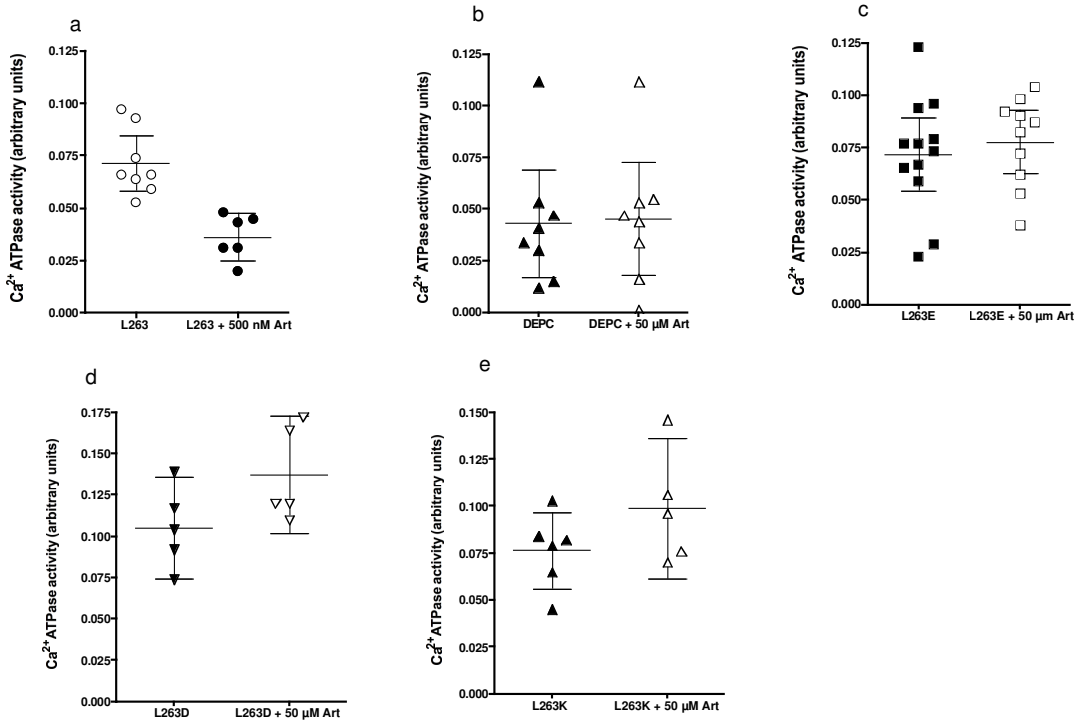
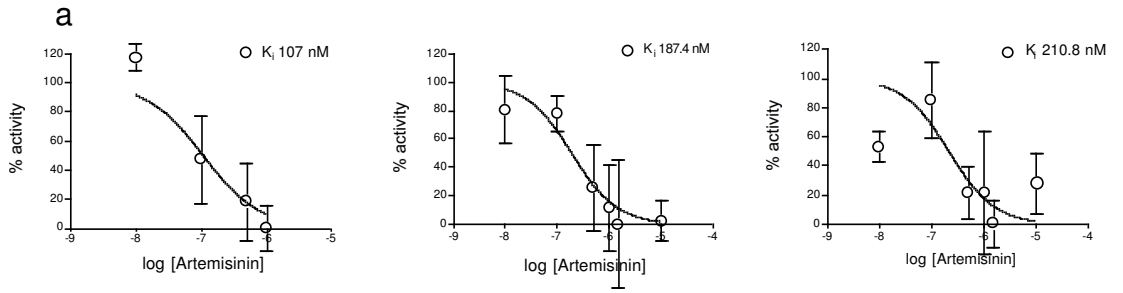
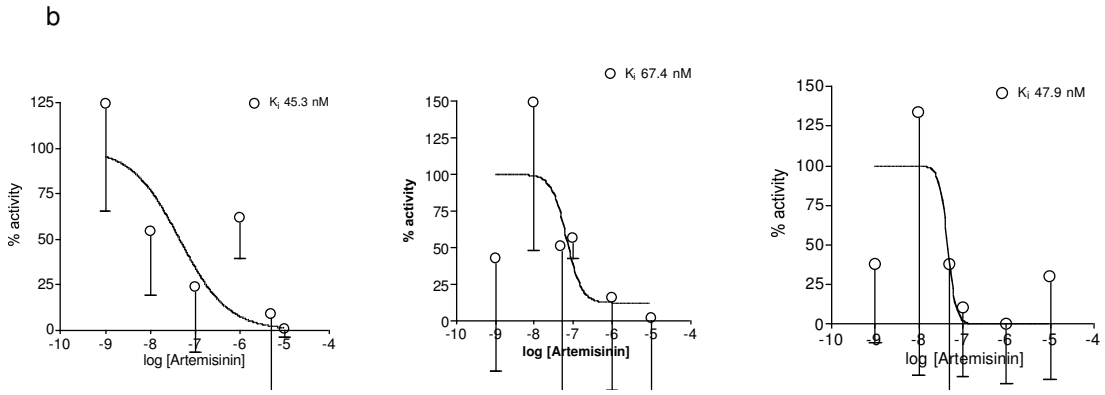


Supplementary Figure 1. Ca^{2+} -ATPase activities of control and artemisinin-resistant mutants of PfATP6 assayed in *Xenopus laevis* oocyte membranes. (**a**) Activity of wild-type PfATP6 without and with artemisinin (Art, 500 nM). Ca^{2+} -ATPase activity is inhibited by 500 nM artemisinin (mean ± s.e.m of 0.072 ± 0.0056 to 0.036 ± 0.0044; p = 0.0005, giving a mean difference value (95% CI for difference) of 0.0352 (0.019 to 0.052). (**b**) Activity of membrane preparations from oocytes injected with water (endogenous Ca^{2+} -ATPase activity) without and with artemisinin (50 µM); no inhibition of basal Ca^{2+} -ATPase activity by artemisinins (control mean ± SEM = 0.043±0.01 compared with 0.045 ± 0.02 Units; *P* = 0.89). (**c**) Activity of an L263E mutation without and with artemisinin (50 µM), showing no inhibition of activity even in the presence of 50 µM artemisinin (mean ± SEM = 0.072±0.008 compared with 0.07 ± 0.0079; p = 0.58). (**d**) Activity of an L263D mutant without and with artemisinin (50 µM); mean ± s.e.m. Ca^{2+} -ATPase activity before and after artemisinin are 0.11 ± 0.01 and 0.14 ±0.013 (*P* = 0.094). (**e**) activity of an L263K mutant without and with artemisinin (50 µM), mean ± SEM Ca^{2+} -ATPase activity before and after artemisinin are 0.076 ± 0.008 and 0.099 ± 0.014 (*P* = 0.17). Data in (**b**) were not presented in the original paper. Data from (**d-e**) are from one representative experiment, whereas results from more than one experiment were reported previously.

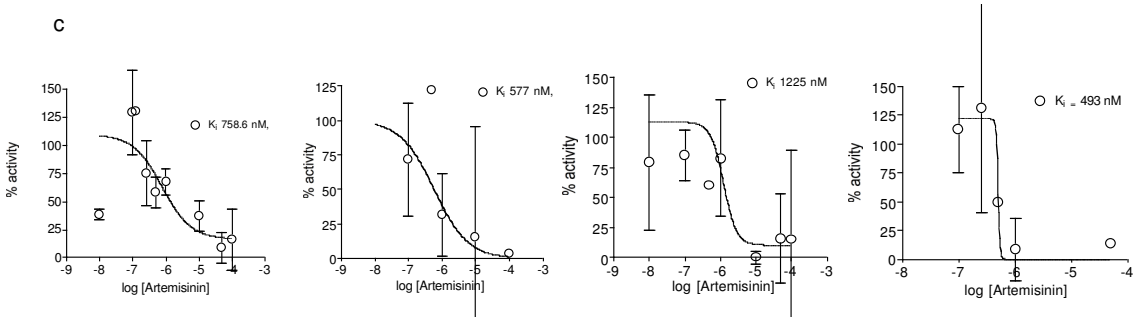
Supplementary Figure 2. Inhibition curves of PfATP6 and mutants with artemisinin or artemisone; the original data were reanalyzed with inclusion of some data and standard analytical procedure (see Suppl Methods). Results from independent experiments are shown in each graph, displayed as Ca^{2+} -ATPase activity in the presence of an artemisinin inhibitor, normalised with respect to values that are obtained without presence of inhibitor.



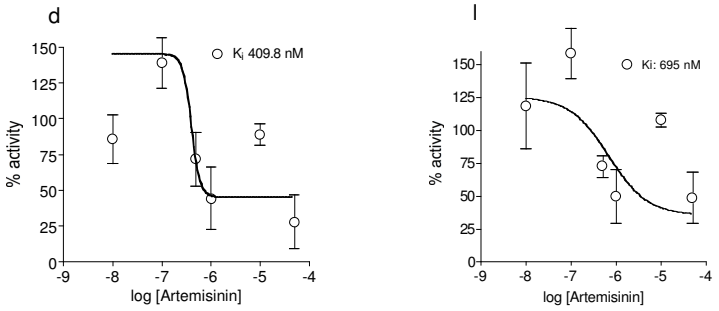
(**a**) Inhibition of PfATP6 Ca^{2+} -ATPase activity by artemisinin with data from 3 independent experiments.



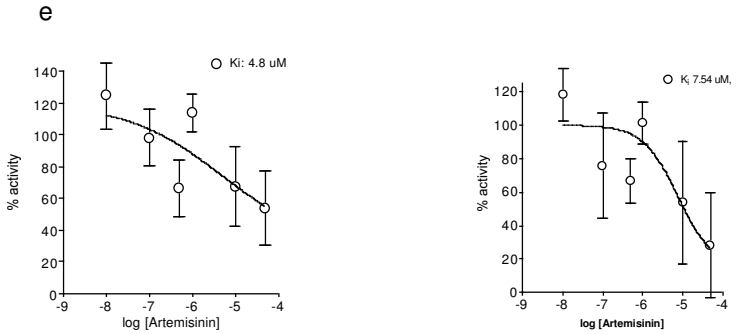
(**b**) Inhibition of PfATP6L263A by artemisinin, with inclusion of 2 possible outlying values (at 1nM). Results from 3 experiments are presented here (4 experiments were analyzed in the original paper).



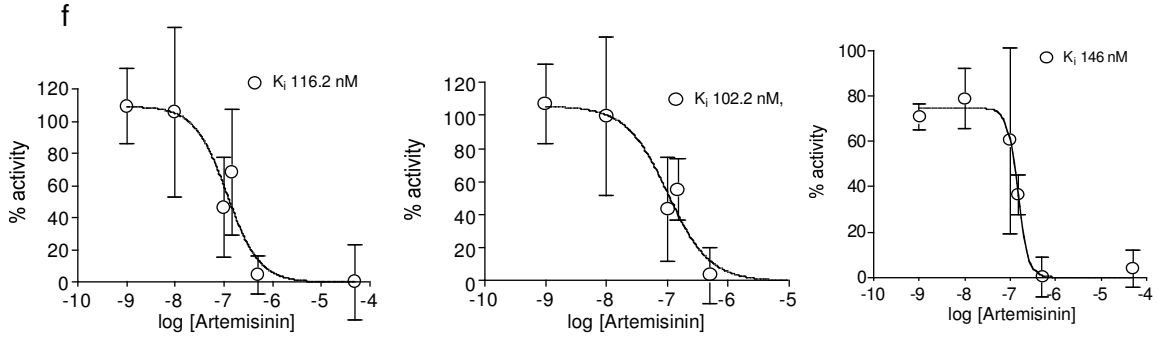
(**c**) Inhibition of PfATP6L263S by artemisinin, with inclusion of possible outlying values. Only the second graph was modelled with constraints.



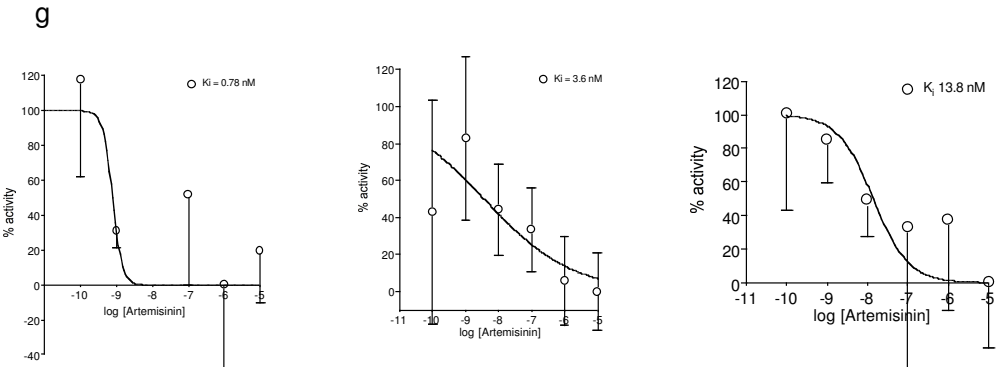
(**d**) Inhibition of PfATP6 L263Q by artemisinin. For modelling, constraints were 120 for Top and 40 for Bottom because of incomplete inhibition at high concentrations.



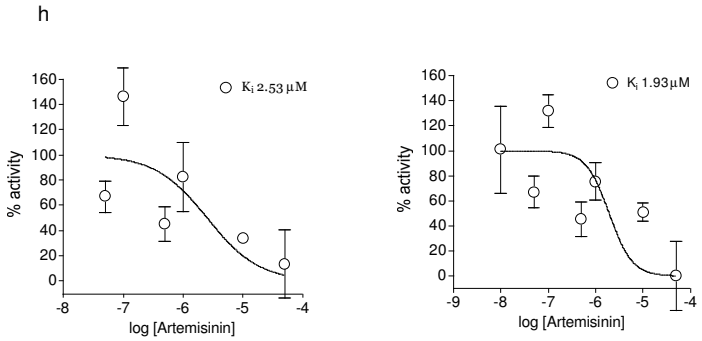
(**e**) Inhibition of F264L PfATP6 by artemisinin. Data could not be retrieved for one experiment. For modelling, constraints were 100 for Top and 15 for Bottom because of incomplete inhibition at high concentrations.



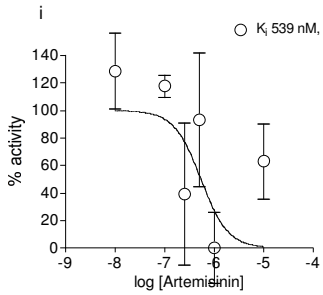
(**f**) Inhibition of PfATP6 I89T by artemisinin modelled without constraint for Top.



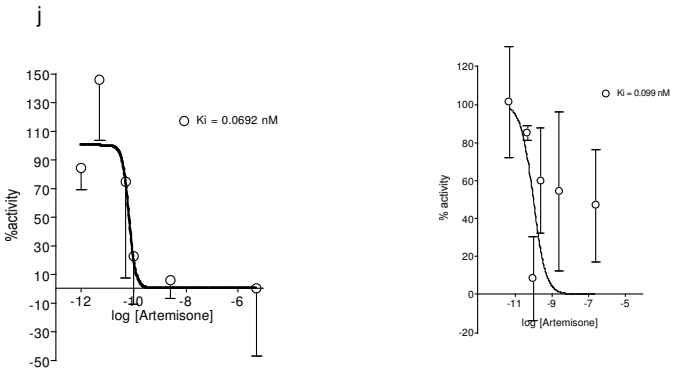
(**g**) Inhibition of PvSERCA by artemisinin. Analysis includes possible outlying values and correction of 2 incorrect values compared with previously reported results.



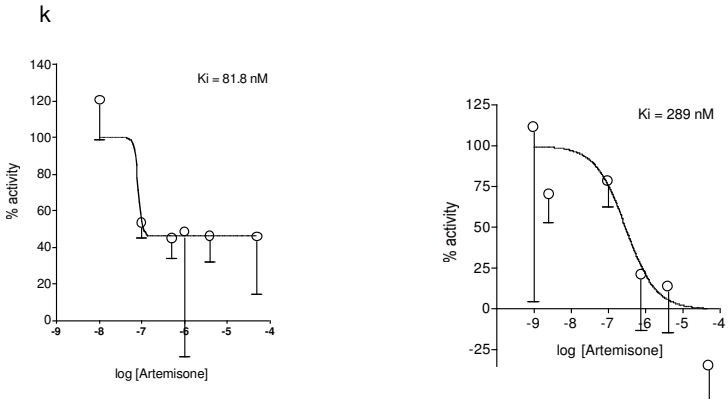
(**h**) Inhibition of PbSERCA by artemisinin. One set of experiments could not be modelled after reanalysis.



(**i**) Inhibition of SERCA1 E255L mutant by artemisinin. One set of data could not be retrieved, and one could not be reanalyzed.



(**j**) Inhibition of PvSERCA by artemisone. One set of data could not be retrieved.



(**k**) Inhibition of PbSERCA by artemisone. One set of data could not be reanalyzed.

Supplementary Methods

Inhibitory constants were derived in Prism (v4.0a, GraphPad Software, Inc.) using a sigmoidal dose-response equation (Y = Bottom+(Top-Bottom)/(1+10^((LogEC50-X)*HillSlope)), where X is the logarithm of the concentration of the inhibitor and Y is the normalised activity of the Ca^{2+} -ATPase activity being assayed. Constraints are 100 for Top and 0 for Bottom unless indicated.

Supplementary Table

PfATP6 wild type

	Experiment 1							Experiment 2				Experiment 3				Experiment 4			
Log [Artemisinin]	% Activity							Log [Artemisinin]	% Activity			Log [Artemisinin]	% Activity			Log [Artemisinin]	% Activity		
-8	101.1407	117.1103	133.0798					-8	32.87671	106.8493	101.3699	-8	49.65035	37.06294	72.72727				
-7	64.65753	-10.9589	87.67123					-7	90.41096	90.41096	52.05479	-7	85.14851	129.703	40.59406				
-6.30103	-35.61644	43.28767	46.57534					-6.30103	52.05479	60.27397	-35.61644	-6.30103	-1.069519	56.68449	8.556149				
-6	21.29278	-31.17871	9.885932					-6	16.43836	-43.83562	60.27397	-6	-30.69307	-9.90099	105.9406				
								-5.823909	86.66666	-66.66666	-20	-5.823909	14.97326	-33.15508	18.18182				
								-5	26.66667	3.333333	-23.33333	-5	53.84615	-13.28671	43.35664				

PfATP6 L263A

Log [Artemisinin]	% Activity							Log [Artemisinin]	% Activity			Log [Artemisinin]	% Activity						
-9	236.9758	39.54699	96.93909					-9	-93.07	174.2574	45.54456	-9	31.90633	126.6453	-45.07				
-8	124.6914	20.98765	17.69547					-8	290.266	201.7699	-46.018	-8	121.3382	-146.9136	426.17				
-7	59.66263	-47.90368	59.66263					-7.30103	-120	-8	280	-7.30103	-197.4	171.832	138.266				
-6	122.1916	16.59014	41.84267	67.42081				-7	56	32	80	-7	-36.27911	-29.56585	97.98602				
-5.30103	79.22015	-82.12933	30.32637					-6	-102.97	114.8515	35.64357	-6	31.90633	43.7487	-74.67507				
-5	4.526749	6.17284	-8.641975					-5	-37.16814	-143.3628	184.0708	-5	5.50221	151.8214	-67.65737				

PfATP6 L263S

Log [Artemisinin]	% Activity							Log [Artemisinin]	% Activity			Log [Artemisinin]	% Activity			Log [Artemisinin]	% Activity		
-8	33.97293	43.186						-8	157.0858	209.4144	247.4716	-8	124.3526	-32.58652	145.9994	-7	132.1691	164.3382	40.25735
-7	125.757	203.028	27.88041	161.8168				-7	96.62174	127.0261	-8.104456	-7	125.5499	74.4501	55.86835	-6.60206	-25.64433	128.9948	290.0773
-6.90309	130.3068	130.3068						-6.60206	-133.0572	39.71545	63.82927	-6.60206	192.2182	369.5609	57.43775	-6.30103		49.25214	
-6.60206	9.952606	17.06161	162.7962	38.38863	60.36807	162.9448		-6.30103	138.0572	171.3572	57.18567	-6.30103	135.3321	24.28831	19.24086	-6	17.27941	49.44853	-42.46323
-6.30103	50.86357	79.52649	95.9053	46.76887	18.10596			-6	-27.82916	53.37995	68.41868	-6	167.6462	-0.1163514	81.05907	-5		-90.07732	58.11856
-6	48.05195	67.53247	87.01299					-5	-100.9485	168.3225	-20.56909	-5	0.1231041	-9.16777	9.413979	-4.30103	-14.85043	86.6453	-30.87607
-5	69.51219	18.29268	40.2439	-3.658537	62.19512			-4.30103	-41.8871	-18.23925	-35.13057	-4.30103	84.85766	4.098526	-41.32849				
-4.30103	-24.97743	-24.97743	39.82568	-1.833465	12.05292	53.71206		-4	38.34123	-42.86789	14.27927	-4	-63.15528	-56.06157	163.8434				
-4	76.08117	63.41825	4.324666	16.98758	-80.09475														

PfATP6 L263Q

Log [Artemisinin]	% Activity			Log [Artemisinin]	% Activity		
-8	73.35	64.46	119.55	-8	92.58	79.38	183.31
-7	122.32	120.46	174.4	-7	139.48	139.48	196.91
-6.30103	66.69	41.9	106.97	-6.30103	56.81	77.38	84.23
-6	12.94	34.26	85.79	-6	16.69	44.74	87.63
-5	79.54	103.72	83.26	-5	98.21	108.97	116.15
-4.30103	14	4.71	65.14	-4.30103	36.24	22.54	86.98

PfATP6 F264L

Log [Artemisinin]	% Activity			Log [Artemisinin]	% Activity		
-8	83.24	148.99	142.55	-8	87.39	136.41	130.81
-7	73.81	133.86	87.86	-7	14.97	119.73	92.52
-6.30103	101.44	55.36	42.4	-6.30103	93.03	58.94	48.09
-6	114.18	134.81	93.55	-6	90.2	126.61	87.39
-5	117.28	42.4	42.4	-5	127.11	9.36	24.85
-4.30103	11.19	91.69	58.47	-4.30103	-34.01	65.31	53.06

PfATP6 I89T

Log [Artemisinin]	% Activity				
-9	188.7574	82.24852	135.503	76.33136	64.49704
-8	37.37236	4.697073	208.9176	257.9306	21.03472
-7	42.02898	46.48829	108.9186	100	-64.99442
-6.823909	1.442308	181.7308	44.71154	44.71154	
-6.30103	-31.86813	4.095904	40.05994	16.08392	-3.896104
-4.30103	33.95087	38.78373	-24.0435	33.95087	-82.03786

Log [Artemisinin]	% Activity				
-9	191.6943	91.12426	65.17856	61.87922	124.3069
-8	91.00367	151.4366	-13.03862	253.5126	14.41416
-7	68.26408	20.66146	138.9998	42.21062	-53.48205
-6.823909	84.4687	42.74481	49.81517	102.8575	-3.667214
-6.30103	-54.4497	45.85186	10.7245	22.63571	-8.598054
-4.30103	51.88929	28.44377	-41.76278	6.630866	-44.89801

Log [Artemisinin]	% Activity			
-9	72.71925	59.88125	79.13825	
-8	81.79612	100	54.49029	
-7	-9.01039	134.0658	55.71453	
-6.823909	21.11651	51.45631	36.28641	
-6.30103	14.83563	-15.82354	1.209334	
-4.30103	-4.308754	11.73875		

PvSERCA

Log [Artemisinin]	% Activity		
-11	288.6647	128.7144	255.8783
-10	95.89794	223.0618	34.36706
-9	38.76926	11.55556	42.17097
-7	155.1945	-8.608564	9.196118
-6	110.2051	-83.69222	-25.86318
-5	80.41485	-15.73044	-5.047627

Log [Artemisinin]	% Activity		
-10	84.26966	-76	120.2247
-9	123.1088	-5.42461	130.1825
-8	-3.83018	59.6216	76.92663
-7	77.18587	20.15057	3.039982
-6	-17.98601	53.35437	-17.98601
-5	42.31657	-21.13521	-21.13521

Log [Artemisinin]	% Activity		
-10	207.077	7.863937	87.54918
-9	71.91327	133.7041	49.44388
-8	72.48212	69.42457	5.216168
-7	-17.0377	226.9984	-111.6639
-6	142.1301	-37.62498	7.313785
-5	-43.70452	78.5972	-34.53189

PbSERCA

Log [Artemisinin]	% Activity		
-7.301	57.66129	91.93549	51.6129
-7	184.0108	148.7805	105.4201
-6.301	21.74985	43.93099	69.809
-6	46.39557	136.3524	64.87985
-5	34.95935		32.24932
-4.301	36.93694	-40.76577	44.81982

Log [Artemisinin]	% Activity		
-8	168.5834	81.37803	53.21797
-7	141.5032	100.5685	111.9393
-6.301	100	61.65192	43.9528
-5	68.66058	59.57669	58.6683
-4.301	63.13364	10.9063	-115.0538

SERCA1 E255L

Log [Artemisinin]	% Activity		
-8	155.1506	73.62363	157.5484
-7	123.6635	101.8203	127.304
-6.60206	-9.598615	142.8864	-15.95216
-6.30103	92.05807	177.8309	9.462014
-6	4.085939	-46.26894	42.45156
-5	18.08799	58.13386	112.7419

PvSERCA

Log [Artemisone]	% Activity		
-12	76.088	62.69727	114.3472
-11.3	87.29675	227.0325	122.8659
-10.3	26.50974	207.5727	-9.702844
-10	88.91353	-15.29933	-6.430155
-8.6	-0.631076	30.06993	-12.57036
-5.3	-87.61726	76.12144	13.01382

Log [Artemisone]	% Activity		
-11.3	144.4551	113.2164	45.93296
-10.3	91.39168	78.00095	85.6528
-10	42.35033	-33.03769	15.74279
-9.6	64.85249	9.468527	105.3254
-8.6	133.0285	39.02439	-9.247968
-6.6	-11.73856	84.38063	67.55978

PbSERCA

Log [Artemisone]	% Activity		
-8	100	163.9	97.22
-7	69.234	41.384	49.217
-6.3	63.53	47.098	24.499
-6	155.56	66.67	-77.78
-5.4	19.626	67.49	51.828
-4.3	69.697	84.078	-16.59

Log [Artemisone]	% Activity		
-9	218.8	4.3	
-8.6	106.34	55.58	50.5
-7	100	87.06	48.22
-6.12	-12.9	55.58	
-5.4	41.75	41.75	-42.4
-4.3	10.14	91.3	-204.35