

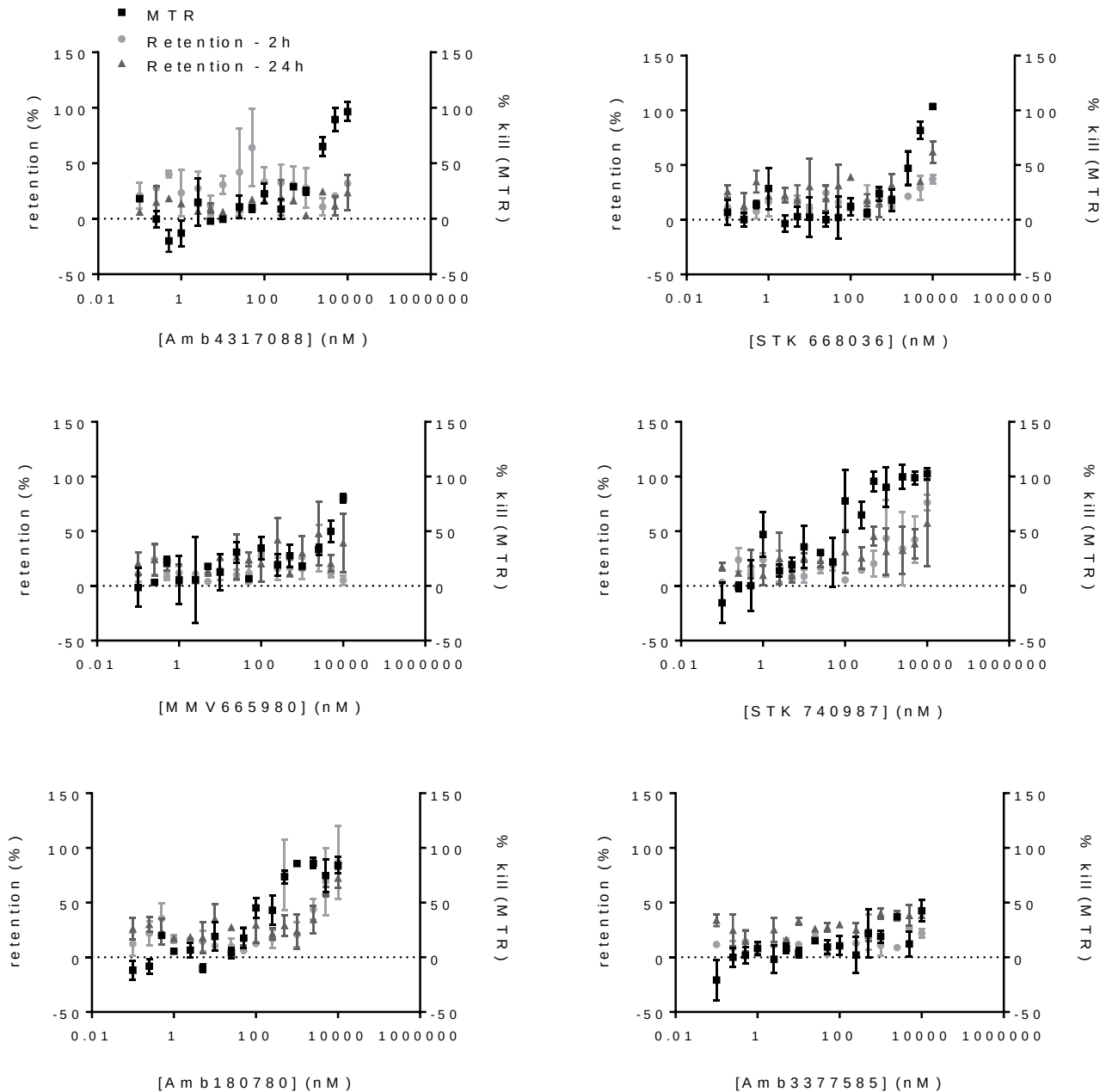


Figure S1: Retention of gametocyte infected RBCs after treatment for 2 h (light grey dots) or 24 h (dark grey triangles) with compound concentrations ranging from 10 μ M to 0.1 nM. Viability of compound treated gametocytes assessed with Mitotracker Red (MTR), shown as black squares. For all tested compounds, any increased retention rates observed coincided with parasite death, therefore no specific RBC deformability change due to compound treatment could be determined.

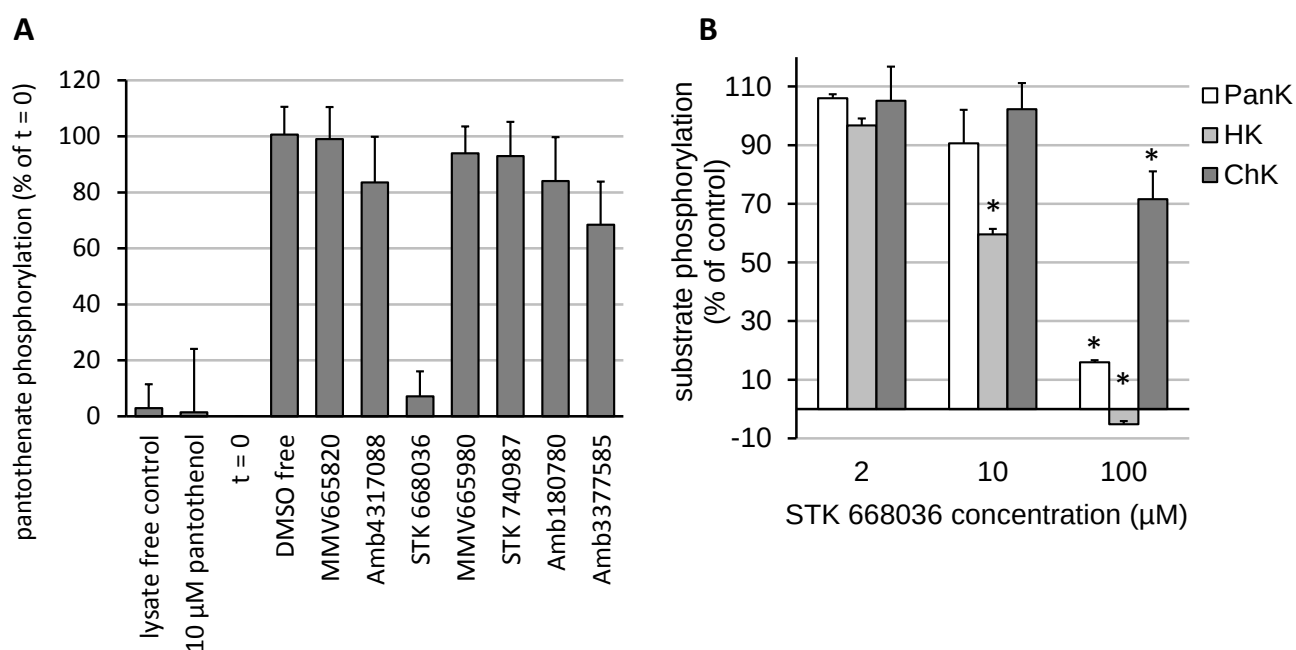
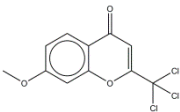
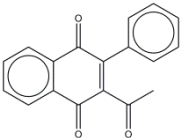
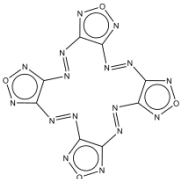
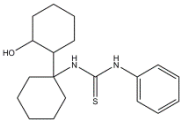
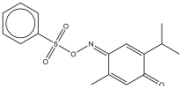
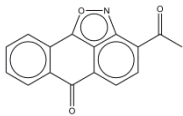
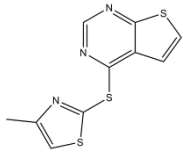


Figure S2: Effect of test compounds on kinase activity using a radiometric assay (n = 2).

(A) Activity of all compounds on PanK at 100 μM; (B) activity of STK668036 on three kinases: PanK, Hexokinase (HK) and Choline kinase (ChK). Asterisks indicate a significant level of inhibition, compared to the control (p < 0.001). Error bars represent standard deviations.

Table S1: Activity overview against *P. falciparum* asexual blood stage forms, early and late stage gametocytes, as well as *T. b. brucei* and *T. cruzi*.

Chemical structure	compound ID	Activity on <i>Trypanosoma</i> spp.			Activity on <i>P. falciparum</i> life stages		
		IC ₅₀ ± SEM (μM) ^a			IC ₅₀ ± SEM (μM) ^a		
		<i>T. b. brucei</i> trypomastigote (SI over HEK293)	<i>T. cruzi</i> amastigote (SI over 3T3 host cell)	<i>T. cruzi</i> trypomastigote (SI over HEK293)	Asexual ^b	Early stage gametocyte	Late stage gametocyte
	MMV665820 / 5534045	2.50 ± 1.66 (nd ^d)	62% ^c (nd ^d)	100% (nd ^d)	0.58 ± 0.08	0.44 ± 0.03	69%
	Amb4317088	0.16 ± 0.01 (15)	4.30 ± 0.27 (2)	0.39 ± 0.03 (6)	1.23 ± 0.06	2.88 ± 0.18	2.24 ± 0.38
	STK 668036	100% (nd ^d)	94% (nd ^d)	2.07 ± 0.46 (6)	1.30 ± 0.08	1.56 ± 0.12	3.53 ± 0.38
	MMV665980/ Amb2368822	100% (nd ^d)	70% (nd ^d)	4.42 ± 0.89 (> 9)	6.31 ± 1.06	7.54 ± 0.39	4.15 ± 0.56
	STK 740987	1.02 ± 0.40 (> 39)	54% (nd ^d)	0.68 ± 0.15 (> 59)	0.38 ± 0.06	0.43 ± 0.06	0.46 ± 0.12

Chemical structure	compound ID	Activity on <i>Trypanosoma</i> spp.			Activity on <i>P. falciparum</i> life stages		
		IC ₅₀ ± SEM (μM) ^a			IC ₅₀ ± SEM (μM) ^a		
		<i>T. b. brucei</i> trypomastigote (SI over HEK293)	<i>T. cruzi</i> amastigote (SI over 3T3 host cell)	<i>T. cruzi</i> trypomastigote (SI over HEK293)	Asexual ^b	Early stage gametocyte	Late stage gametocyte
	Amb3377585	0.20 ± 0.01 (14)	13.30 ± 2.35 (1)	0.74 ± 0.13 (4)	0.15 ± 0.01	0.25 ± 0.01	6.22 ± 2.5
	Amb180780	100% (nd ^d)	21% (nd ^d)	100% (nd ^d)	0.12 ± 0.02	0.06 ± 0.004	0.07 ± 0.01

^aPercent inhibition at the highest concentration tested (40 μM) is given if an IC₅₀ value could not be calculated due to low activity of the compound

^bAs previously reported (1)

^cLow plateau level of CRC curve in cytotoxicity assay

^dAn accurate IC₅₀ value could not be calculated

Abbreviations: nd, not determined; SI, The selectivity indices of compound activity against *T. b. brucei* and *T. cruzi* trypomastigote forms was calculated over HEK293 cytotoxicity [22] and of *T. cruzi* amastigote forms over 3T3 host cell cytotoxicity

1. **Fletcher S, Avery VM.** 2014. A novel approach for the discovery of chemically diverse anti-malarial compounds targeting the Plasmodium falciparum Coenzyme A synthesis pathway. *Malar J* 13:343.

Table S2: Rescue of inhibitor-treated asexual blood stage *P. falciparum*, early stage and late stage *P. falciparum* gametocytes and *T. b. brucei* trypomastigotes by supplementation with CoA pathway intermediates. Percent rescue averages $\pm$ SEM. The rescue experiment was not carried out for inactive compounds (blank spaces). All numeric values are statistically significant ($P < 0.001$)

Enzyme	PanK				PPCS / PPCDC				PPAT / DPCK				Complete pathway			
Growth inhibition rescued by	Panthothenate (competitive inhibitors)				Pantethine				Dephospho CoA				CoA			
stage / species	PfAS	PfEG	PfLG	Tbb	PfAS	PfEG	PfLG	Tbb	PfAS	PfEG	PfLG	Tbb	PfAS	PfEG	PfLG	Tbb
Amb180780	No	No	No		76.2 $\pm$ 7.7	94.2 $\pm$ 1.8	No		99.3 $\pm$ 3.8	104.6 $\pm$ 0.5	95.2 $\pm$ 1.3		113.5 $\pm$ 4.4	111.3 $\pm$ 3.2	105.7 $\pm$ 2.9	
MMV665820/ 5534045	No	No			No	No			88.6 $\pm$ 18.3	98.7 $\pm$ 1.9			113.3 $\pm$ 12.3	98.8 $\pm$ 3.0		
Amb4317088	No		No	No	No		No	No	77.6 $\pm$ 5.5		107.9 $\pm$ 1.7	84.7 $\pm$ 2.6	84.5 $\pm$ 7.7		128.5 $\pm$ 2.6	85.0 $\pm$ 6.9
STK 668036	No				No				81.7 $\pm$ 5.4				82.9 $\pm$ 4.8			
Amb2368822/ (MMV665980)	No				No				46.0 $\pm$ 2.6				54.4 $\pm$ 2.5			
STK 740987	No	No	No	No	No	No	No	No	88.9 $\pm$ 6.7	23.2 $\pm$ 1.2 (46.2 $\pm$ 2.4) ^a	50.3 $\pm$ 0.8	90.3 $\pm$ 5.3	103.9 $\pm$ 3.4	24.1 $\pm$ 0.7 (61.5 $\pm$ 1.0) ^a	53.1 $\pm$ 0.5	69.6 $\pm$ 10.6
Amb3377585	No	No		34.2 $\pm$ 1.3	No	No		No	38.7 $\pm$ 4.7	89.5 $\pm$ 0.3		100.4 $\pm$ 1.4	89.3 $\pm$ 2.6	103.4 $\pm$ 1.8		95.0 $\pm$ 4.2

Abbreviations: PfAS, *Plasmodium falciparum* asexual blood stages; PfEG, *P. falciparum* early stage gametocytes (stage I-III); PfLG, *P. falciparum* late stage gametocytes (stages IV-V); Tbb, *Trypanosoma brucei brucei*

^apercent rescue at the compound's IC₅₀ is shown in brackets

Table S3: Activity of MMV000570 against *Trypanosoma* spp., cytotoxicity against a panel of cell lines and selectivity indices (A); the activity of MMV000570 against *P. falciparum* asexual stages and early stage gametocytes could not be rescued by CoA pathway metabolites; percent rescue averages ± SEM (B).

A

Activity on <i>Trypanosoma</i> spp.; IC ₅₀ [μM] ± SEM†			Cell line specific cytotoxicity; IC ₅₀ [μM] ± SEM†				
<i>T. b. brucei</i> trypomastigote (SI over HEK293)	<i>T. cruzi</i> amastigote (SI over 3T3 host cell)	<i>T. cruzi</i> trypomastigote (SI over HEK293)	HEK293*	3T3	MCF7	MDA-MB-231	PC-3
5.85 ± 1.11 (~ 6.8)	96% (ND)	100% (ND)	~ 40	> 40	96%	90%	98%

† Percent inhibition at the highest concentration tested (40 μM) is given if an IC₅₀ value could not be calculated due to low activity of the compound

SI: The selectivity indices of compound activity against *T. b. brucei* and *T. cruzi* trypomastigote forms was calculated over HEK293 cytotoxicity and of *T. cruzi* amastigote forms over 3T3 host cell cytotoxicity.

* As previously reported (1)

B

enzyme		PanK					PPCS / PPCDC					PPAT / DPCK					Complete pathway				
Growth inhibition rescued by		Panthothenate					Pantethine					Dephospho CoA					CoA				
stage‡ (replicate #)	PfAS (1)	PfAS (2)	PfAS (3)	PfEG (1)	PfEG (2)	PfAS (1)	PfAS (2)	PfAS (3)	PfEG (1)	PfEG (2)	PfAS (1)	PfAS (2)	PfAS (3)	PfEG (1)	PfEG (2)	PfAS (1)	PfAS (2)	PfAS (3)	PfEG (1)	PfEG (2)	
tested at IC ₅₀	3.5 ±	-0.6 ±		4.3 ±	28.6 ±	3.5 ±	1.4 ±		5.1 ±	20.9 ±	2.3 ±	2.7 ±		15.6 ±	59.0 ±	3.8 ±	4.7 ±		17.5 ±	61.3 ±	
concentration	1.3	0.8		0.4	3.8	0.5	1.5		0.2	3.2	0.4	1.4		0.5	2.4	0.8	3.4		1.0	2.1	
tested at IC ₈₀	3.1 ±	2.2 ±	2.3 ±	5.2 ±		2.1 ±	1.2 ±	0.1 ±	5.5 ±		4.6 ±	6.3 ±	1.2 ±	26.0 ±		6.4 ±	8.1 ±	-1.6 ±	26.7 ±		
concentration	1.4	1.3	2.3	1.1		0.8	0.8	1.6	1.2		0.9	2.4	1.3	1.3		1.2	2.1	1.3	1.5		

‡ PfAS: *Plasmodium falciparum* asexual blood stages; PfEG: *P. falciparum* early gametocytes (stage I-III)

1. **Fletcher S, Avery VM.** 2014. A novel approach for the discovery of chemically diverse anti-malarial compounds targeting the *Plasmodium falciparum* Coenzyme A synthesis pathway. *Malar J* **13**:343.

Table S4: Cytotoxicity of test compounds on human cancer cell lines in comparison with reference cell lines and antiparasmodial activity.

Compound ID	Asexual <i>P. falciparum</i> ; IC ₅₀ [μM] ± SEM*	Cell line specific cytotoxicity; IC ₅₀ [μM] ± SEM†				
		HEK293* (SI)	3T3	MCF7	MDA-MB-231	PC-3
MMV665820 / 5534045	0.58 ± 0.08	3.6 ^a ± 0.24 (ND)	0.47 ^a	0.27 ^a ± 0.05	0.43 ^a	0.35 ^a ± 0.01
Amb4317088	1.23 ± 0.06	2.33 ± 0.12 (1.9)	8.6 ± 0.96	4.6 ± 0.16	0.87 ± 0.04	1.4 ± 0.06
STK 668036	1.30 ± 0.08	11.92 ± 1.32 (9.2)	> 20	5.5 ± 0.21	> 4	3 ± 0.12
MMV665980/ Amb2368822	6.31 ± 1.06	> 40 (> 6)	> 40	> 40	> 40	> 20
STK 740987	0.38 ± 0.06	> 40 (> 105)	> 20	> 40	> 40	> 20
Amb3377585	0.15 ± 0.01	2.75 ± 0.18 (18.3)	13.4 ^a ± 1.95	9.1 ± 0.62	5.6 ± 0.33	5.5 ± 0.64
Amb180780	0.12 ± 0.02	~ 40 (~ 333)	> 40	> 40	> 40	> 40

IC₅₀: inhibitory concentration 50%

SEM: standard error of mean

SI: selectivity index.

† the highest concentration resulting in < 50 % inhibition is given if the inhibition plateau was not reached by 40 μM and an IC₅₀ value could therefore not be accurately calculated; it is inferred that the actual IC₅₀ must be higher than the given values of < 50% inhibition.

* As previously reported (1).

^a Low plateau level of concentration-response curve does not allow determination of accurate IC₅₀.

ND: could not be determined.

1. **Fletcher S, Avery VM.** 2014. A novel approach for the discovery of chemically diverse anti-malarial compounds targeting the Plasmodium falciparum Coenzyme A synthesis pathway. *Malar J* **13**:343.

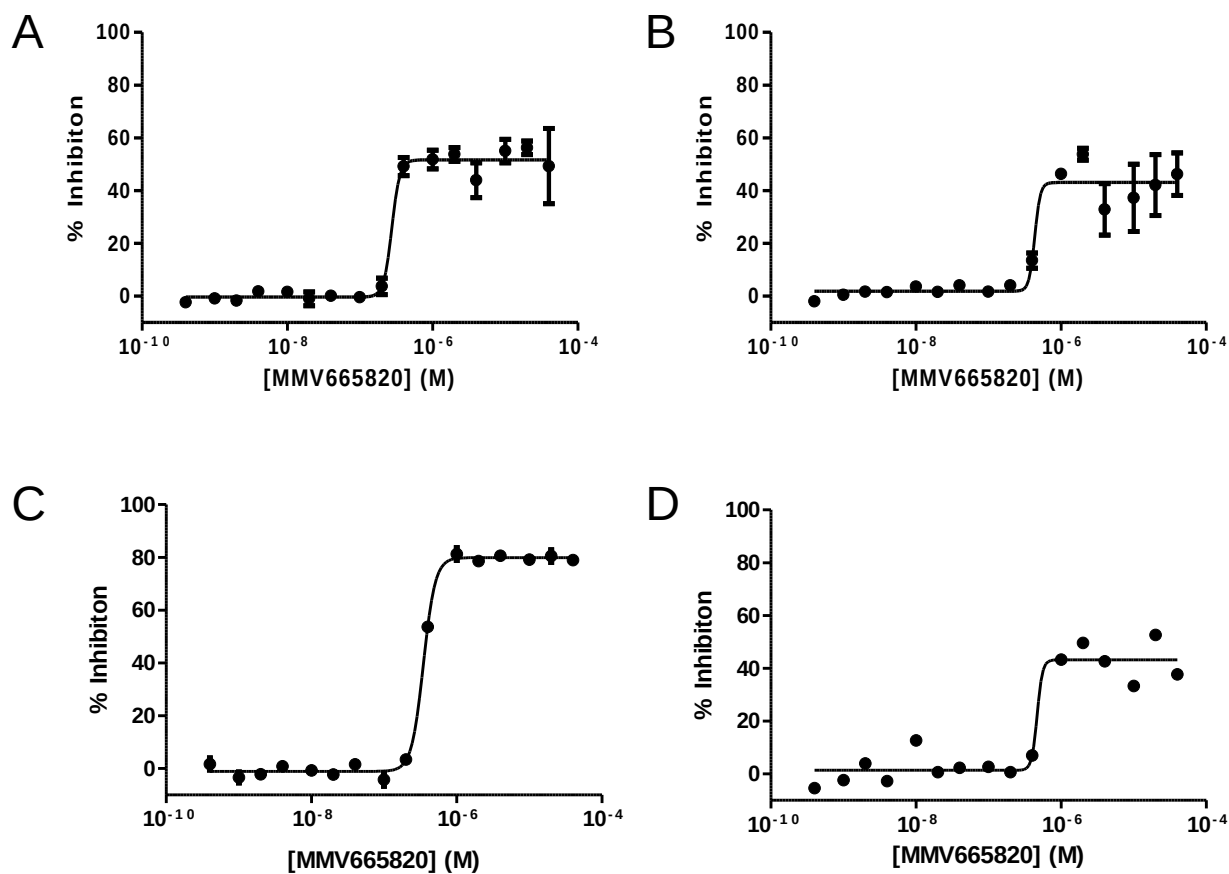


Figure S3: Concentration response curves displaying cytotoxic effect of MMV665820 on the four mammalian cell lines MCF7 (A), MDA-MB-231 (B), PC3 (C) and 3T3 (D).