Supplementary data

Figure S1. Susceptibility of bloodstream forms of *T. brucei* 427 WT (A) and *L. mexicana* promastigotes (B) or amastigotes (C) to compounds T1 and MS1. The experiments were performed using Alamar blue as described in the Materials and methods section and are representative of at least three independent determinations. T1, filled circles; and MS1, open triangles. The positive control (filled squares) was diminazene aceturate (A) or pentamidine (B and C).

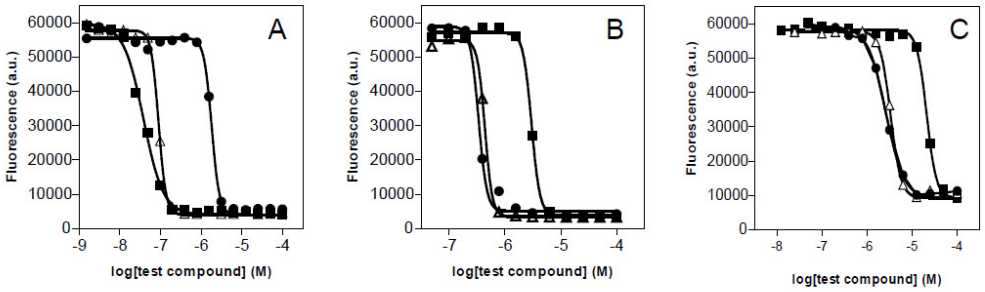


Figure S2. Choline uptake by kinetoplastid parasites. Uptake of 0.25 µM [^{3}H]choline uptake by (A) *T. brucei* 427 bloodstream forms and (B) *L. mexicana* promastigotes over 120 s in the presence (open circles) or absence (filled squares) of 1 mM unlabelled choline was monitored at the indicated incubation times. Assays were performed in triplicate and are representative of four similar experiments. Error bars are SEM.

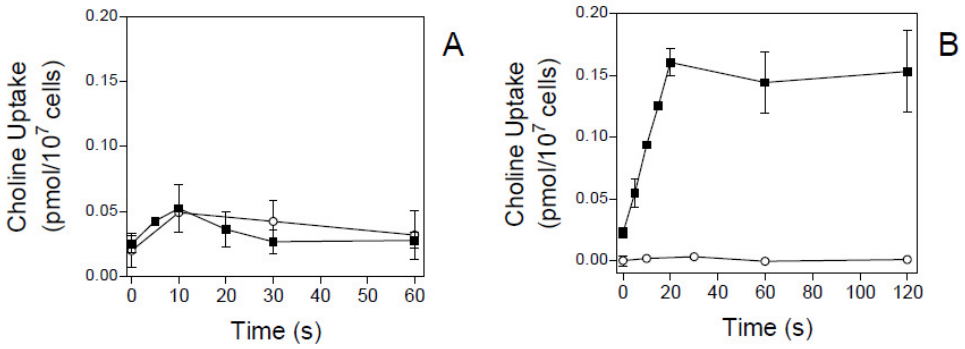
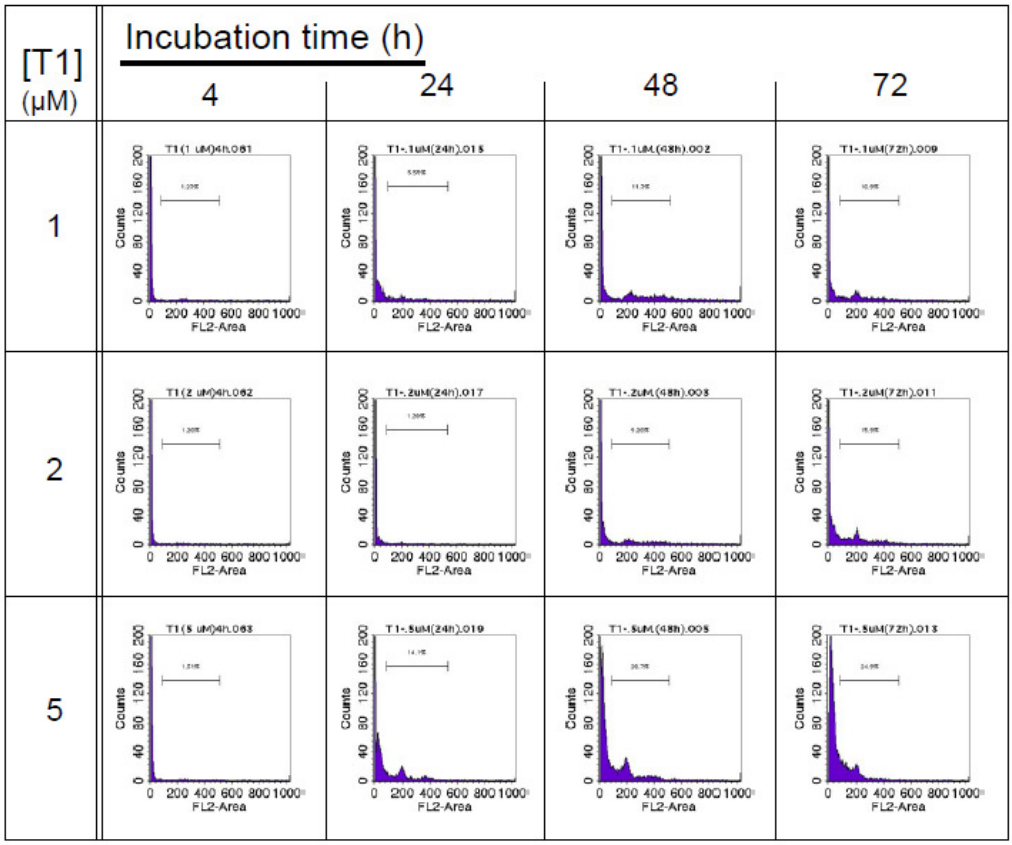
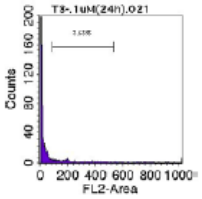
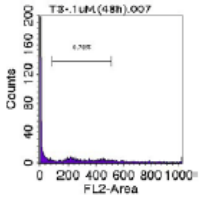
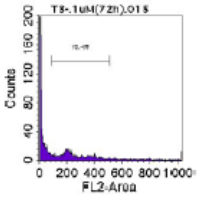
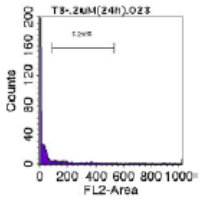
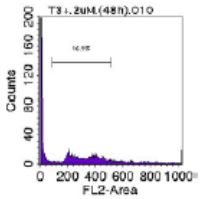
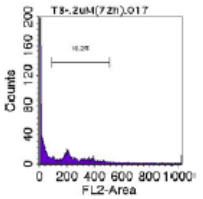
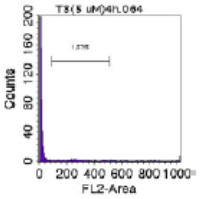
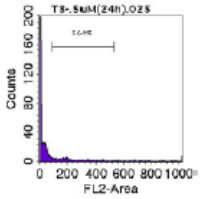
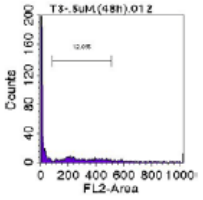
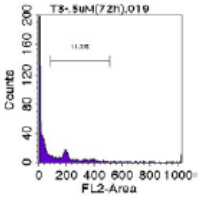


Figure S3. DNA content of PI-permeable *T. b. brucei* after incubation with choline-derived analogues. Cultures treated with various concentrations of the compounds were harvested by centrifugation (10 min, 2500 **g**), stained for 10 min with 5 µg/mL PI at room temperature and in the dark, and analysed by flow cytometry.



[T3] (μM)	Incubation time (h) 4	24	48	72
1				
2				
5				

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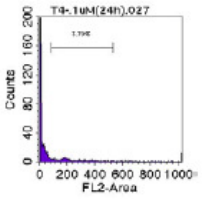
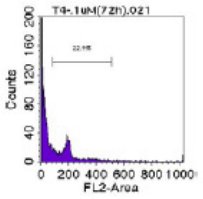
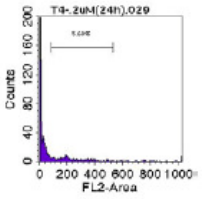
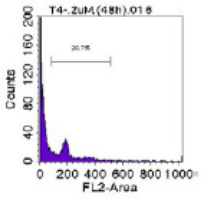
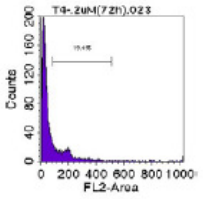
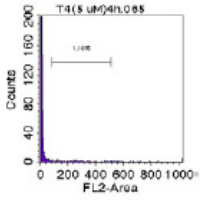
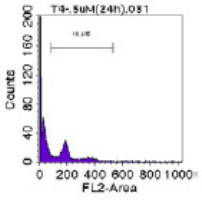
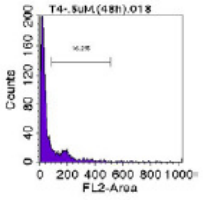
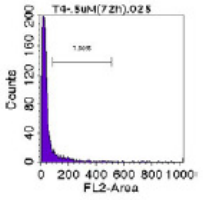
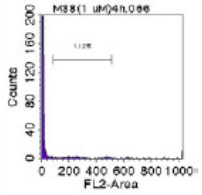
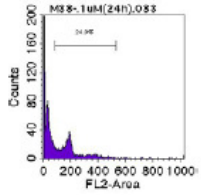
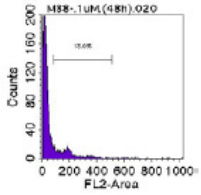
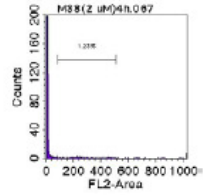
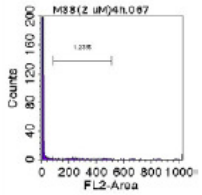
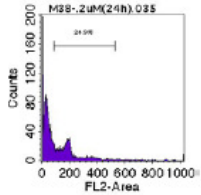
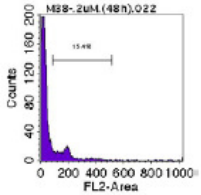
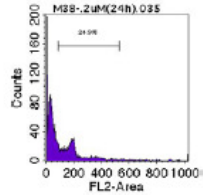
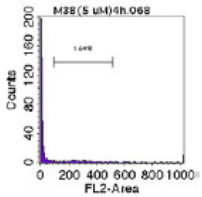
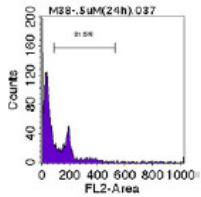
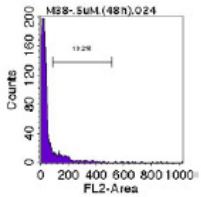
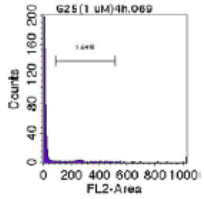
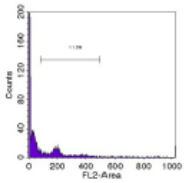
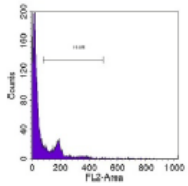
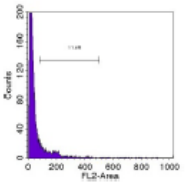
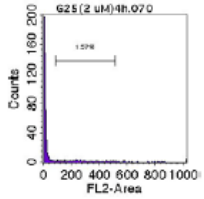
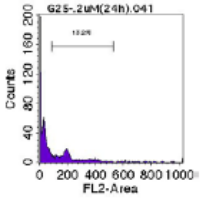
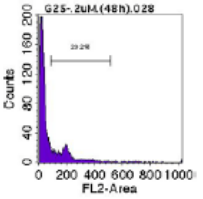
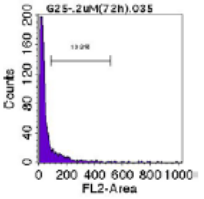
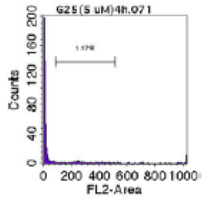
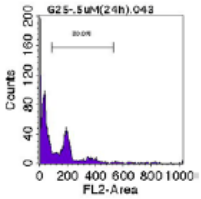
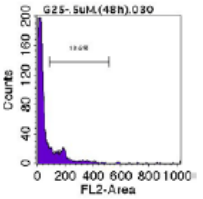
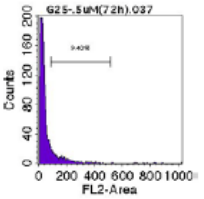
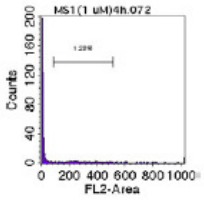
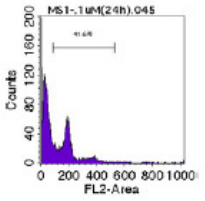
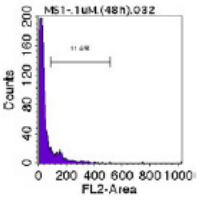
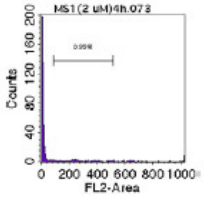
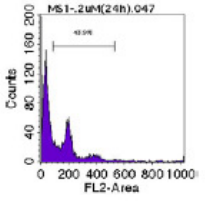
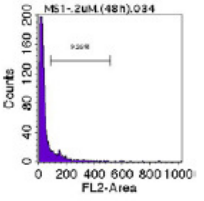
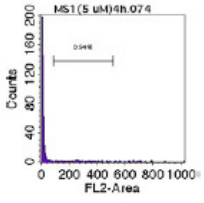
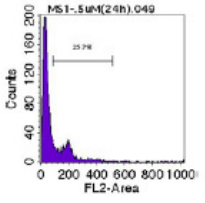
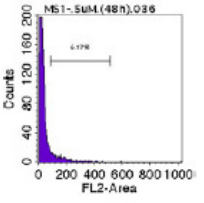
1				
2				
5				

[M38] (μM)	Incubation time (h) 4	24	48	72
1				
2				
5				

[G25] (μM)	Incubation time (h) 4	24	48	72
1				
2				
5				

[MS1] (μM)	Incubation time (h) 4	24	48	72
1				
2				
5				

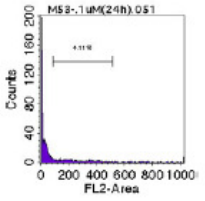
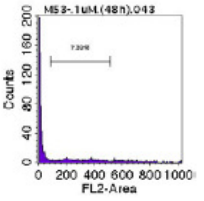
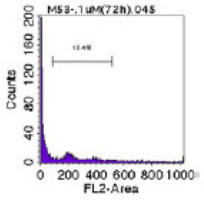
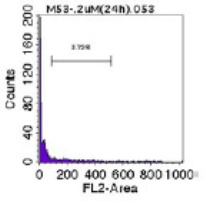
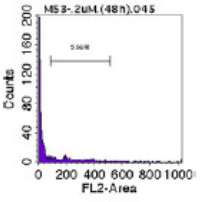
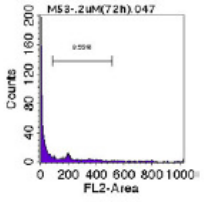
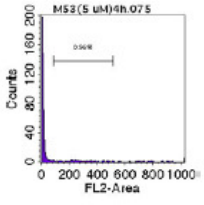
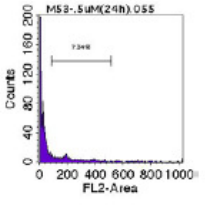
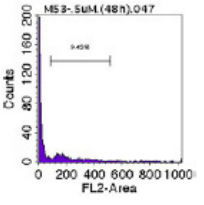
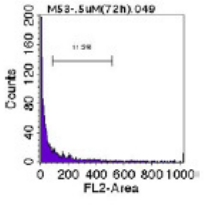
[M53] (μM)	Incubation time (h) 4	24	48	72
1				
2				
5				

Figure S4. Effect of choline-derived analogues on intracellular calcium levels. WT trypanosomes loaded with Fluo-8 were incubated with choline test compound (20 µM) or calcium ionophore A23187 (10 µM) and fluorescence was monitored. The experiment is representative of three identical ones.

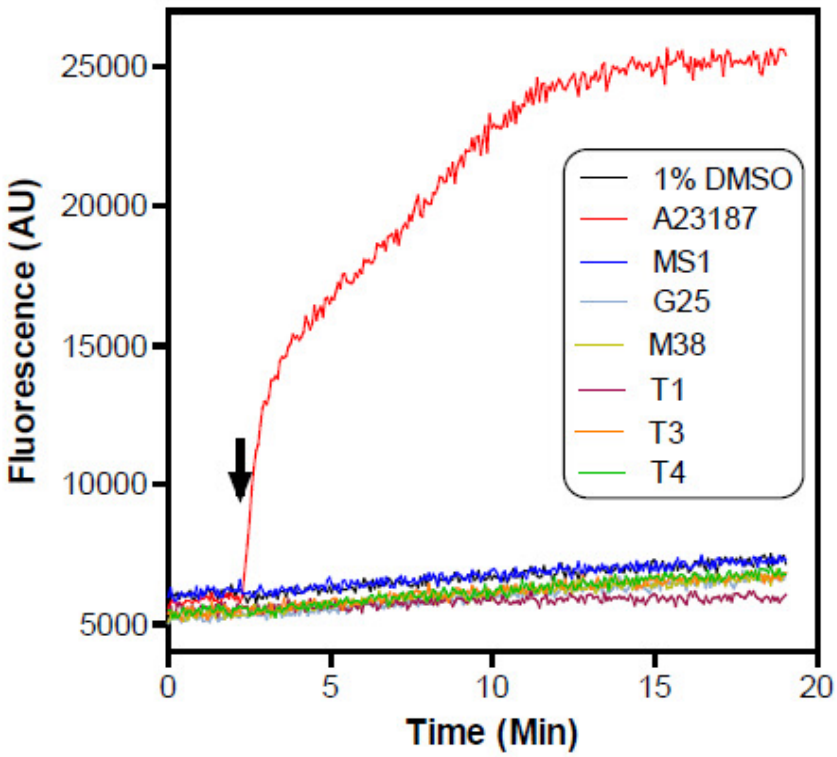
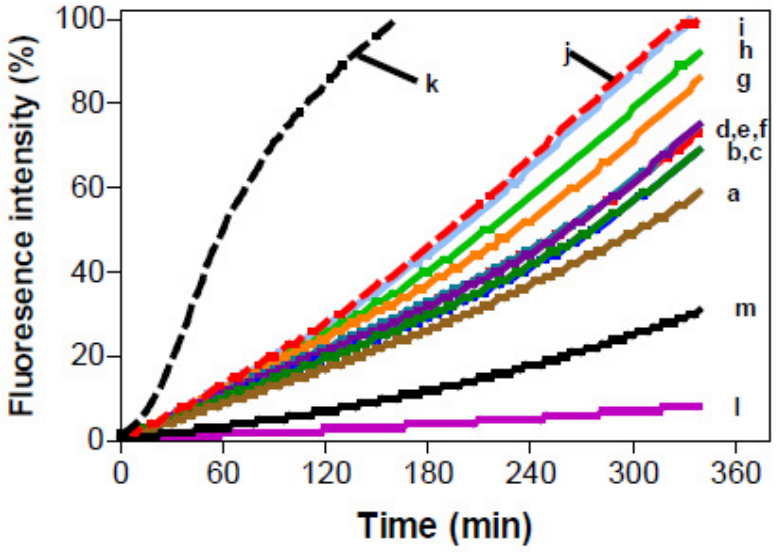


Figure S5. Effect of choline-derived analogues on production of ROS in bloodstream forms of *T. brucei* WT. Parasites at 2×10^6 cells/mL in PBS were cultured in 96-well plates in the presence or absence of 100 μM H_2O_2 and the choline-derived analogues T1 (A) and MS1 (B). Production of ROS was measured using the fluorescent indicator dye DCFH-DH. Choline analogues were at: 50 μM (a); 25 μM (b); 12.5 μM (c); 6.25 μM (d); 3.13 μM (e); 1.56 μM (f); 0.78 μM (g); 0.39 μM (h) and 0.195 μM (i). Other traces represent: parasites incubated without test compound (j); cells incubated with 100 μM H_2O_2 (k); PBS without parasites (l); and 100 μM H_2O_2 without parasites (m). This experiment is representative of two identical experiments performed independently.


A
Fluoresence intensity (%)
Time (min)
0
20
40
60
80
100
0
60
120
180
240
300
360
i
h
g
d,e,f
b,c
a
m
l
j
k

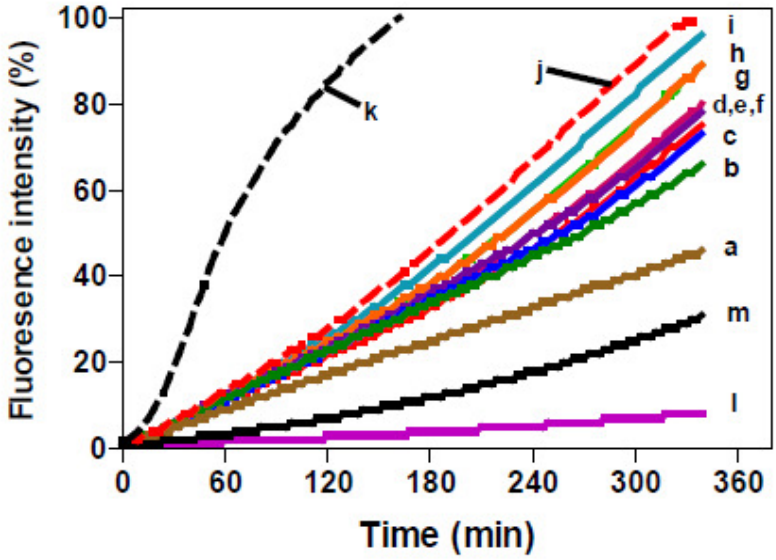

B
Fluoresence intensity (%)
Time (min)
0
20
40
60
80
100
0
60
120
180
240
300
360
i
h
g
d,e,f
c
b
a
m
l
j
k

Table S1. Inhibition constants (K_i) for choline-derived compounds against the *T. b. brucei* diamidine transporters

	HAPT K_i (μM), *n*	LAPT K_i (μM), *n*
Pentamidine[a]	0.036±0.006, 3	56.2±8.3, 3
G25	44±10, 4	560±200, 3
M38	45±4, 3	200±70, 3
T4	210±50, 3	>1000, 2

All experiments were performed in triplicate with bloodstream form *T. b. brucei* 427.

The number of independent repeats is indicated.

[a]Values taken from: De Koning HP. Uptake of pentamidine in *Trypanosoma brucei brucei* is mediated by three distinct transporters. Implications for crossresistance with arsenicals. *Mol Pharmacol* 2001; **59**: 586-92.

Table S2. Flow cytometry results of DNA content of *T. brucei* bloodstream forms after treatment with choline test compounds

Compounds		8 h			24 h		
		2C (%)	4C (%)	≥8C (%)	2C (%)	4C (%)	≥8C (%)
Drug-free control		70.4	25.2	4.4	70.1	25.0	4.9
T3	5 µM	61.0	31.7	7.3	70.9	23.7	5.4
	7.5 µM	64.2	29.8	6.1	66.8	26.5	6.7
	10 µM	63.3	30.8	5.9	65.8	26.9	7.3
T4	5 µM	63.2	29.9	6.9	73.9	21.7	4.5
	7.5 µM	60.4	31.6	7.9	72.6	22.2	5.2
	10 µM	72.2	24.3	3.5	74.1	21.1	4.8
M38	5 µM	76.0	20.2	3.8	83.5	14.4	2.1
	7.5 µM	77.3	19.0	3.7	79.7	16.9	3.5
	10 µM	76.0	20.4	3.6	ND	ND	ND
G25	5 µM	76.4	20.0	3.6	82.5	15.4	2.1
	7.5 µM	77.7	18.7	3.6	81.5	15.8	2.7
	10 µM	75.8	19.6	4.6	ND	ND	ND
MS1	5 µM	75.6	21.1	3.3	ND	ND	ND
	7.5 µM	75.3	21.3	3.5	ND	ND	ND
	10 µM	ND	ND	ND	ND	ND	ND
M53	5 µM	60.3	32.5	7.3	64.0	29.6	6.4
	7.5 µM	65.6	28.3	6.1	57.4	36.2	6.4
	10 µM	76.0	20.6	3.4	60.1	33.5	6.4

ND, not determined because too few cells with intact DNA were observed.