Supplementary material

Activity profile of two 5-nitroindazole derivatives over the moderately drug-resistant *Trypanosoma cruzi* Y strain (DTU TcII): *In vitro* and *in vivo* studies

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Figure S1. Unspecific cytotoxicity

Fig. S1. Cytotoxicity profile of 5-nitroindazole derivatives 16 (A), 24 (B) and BZ (C) over primary cultures of cardiac cells, after 24 and 48 h of treatment at 37 ºC in a humidified atmosphere of 5% CO_2. Bars represent the mean ± SD of the results obtained in three independent assays (n = 3). *Significant differences ($P < 0.05$) with BZ, U-Mann Whitney.

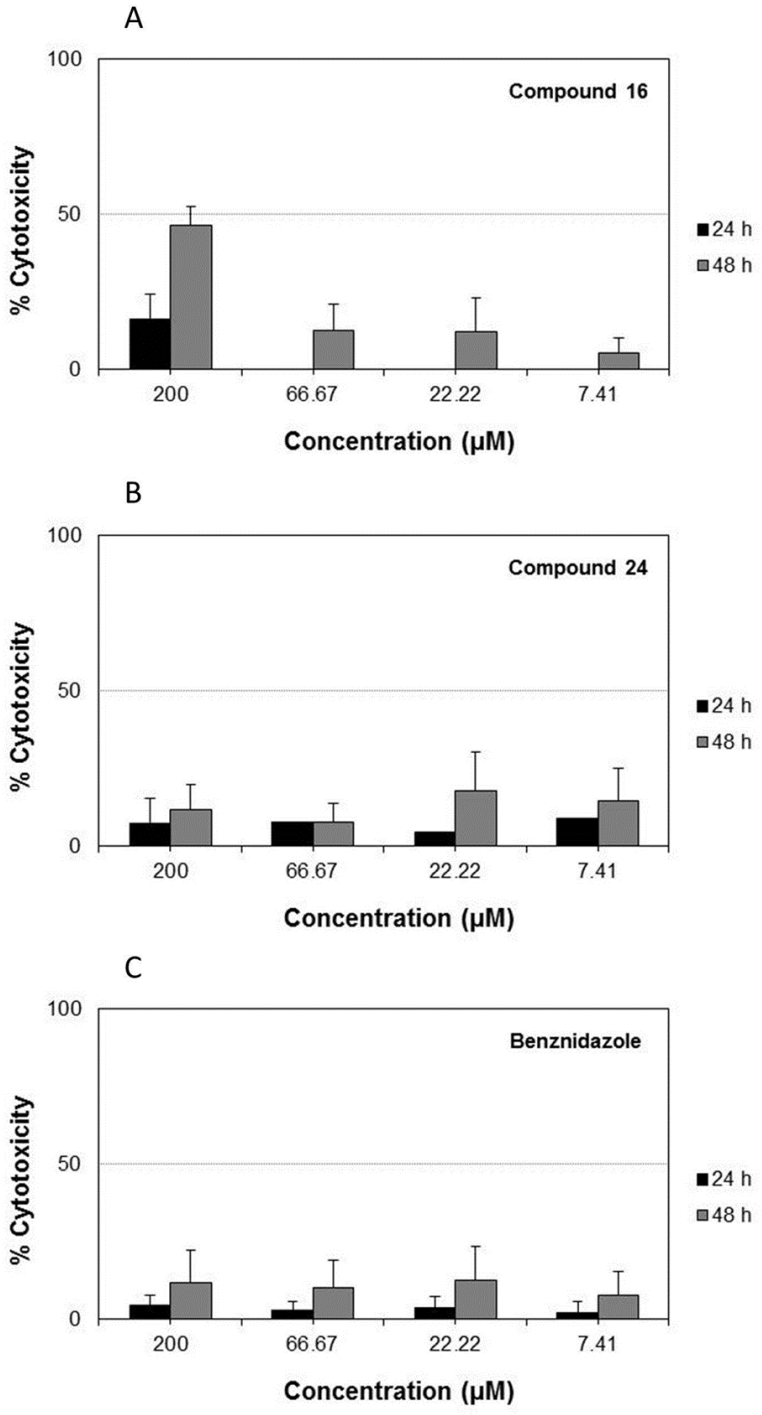

Table S1. Determination of NOAEL

Table S1. Determination of NOAEL *(no-observed-adverse-effect level)* 48 h after concluding treatments. Escalating doses of 5-nitroindazole derivatives 16 and 24 administered every 2 h on day 1, p.o. or ip (0.1 mL/dose), to male Swiss Webster mice (n = 2 per group, two independent assays).

Compound	via	Cumulative dose in mice				NOAEL 48 h
		20 mg kg^{-1}	50 mg kg^{-1}	100 mg kg^{-1}	200 mg kg^{-1}	
16	p.o.	NDE [a]	NDE	NDE	NDE	> 200 mg kg^{-1}
	ip	NDE	NDE	NDE	NDE	> 200 mg kg^{-1}
24	p.o.	NDE	NDE	NDE	NDE	> 200 mg kg^{-1}
	ip	NDE	NDE	NDE	NR [b]	> 100 mg kg^{-1}

[a] NDE: no detectable effects.

[b] NR: not reached due to solubility problems.

Table S2. Biochemical analysis

Table S2. Biochemical analysis performed in serum samples collected from male Swiss Webster mice 48 h after concluding treatments with 5-nitroindazole derivatives 16 and 24. Results are expressed as the mean ± SD (n = 2 per group, two independent assays).

Compound	via	ALT (U/L)	AST (U/L)	Creatinine (mg/dL)	CK (mg/dL)
16	p.o.	62.25 ± 10.25	119.25 ± 42.78	0.10 ± 0	405.00 ± 472.35
		#1 (59; 51)	#1 (163; 136)	#1 (0.1; 0.1)	#1 (752; 726)
		#2 (66; 73)	#2 (74; 104)	#2 (0.1; 0.1)	#2 (36; 106)
	ip	65.50 ± 3.54	140.00 ± 20.51	0.10 ± 0	362.00 ± 277.89
		#1 (50; 86)	#1 (115; 136)	#1 (0.1; 0.1)	#1 (599; 518)
		#2 (70; 56)	#2 (178; 131)	#2 (0.1; 0.1)	#2 (180; 151)
24	p.o.	65.25 ± 6.01	150.00 ± 8.49	0.10 ± 0	1,017.75 ± 134.00
		#1 (ND[a]; 61)	#1 (ND; 156)	#1 (0.1; 0.1)	#1 (1215; 1010)
		#2 (73; 66)	#2 (157; 131)	#2 (0.1; 0.1)	#2 (1021; 825)
	ip	62.00 ± 8.49	161.00 ± 21.21	0.10 ± 0	866.50 ± 550.84
		#1 (56; ND)	#1 (129; 223)	#1 (0.1; 0.1)	#1 (583; 371)
		#2 (66; 70)	#2 (146; ND)	#2 (0.1; 0.1)	#2 (1256; ND)
Ref. values[b]	–	28–132	59–247	0.20–0.80	68–1,070

In brackets, individual values obtained in each experiment.

[a] ND: not determined.

[b] Reference values for healthy mice, provided by ICTB/FIOCRUZ.

* Significant differences ($P < 0.05$) with the respective control group, Kruskal-Wallis.

Figure S2. Ponderal curve analysis I

Fig. S2. Ponderal curve analysis of different groups of male Swiss Webster mice (n = 5) treated with two unique doses of derivatives 16 (A) and 24 (B), administered by using two different vehicles (i.e. 10% DMSO and 20% Trappsol) and given at 5th and 8th dpi, either p.o. or ip. Results are expressed as the mean ± SD. *Significant differences (P < 0.05) with the untreated group, one-way ANOVA. #Significant differences (P < 0.05) with the group of non-infected mice, one-way ANOVA. Note: n = 4 for the group treated with 24 - Trappsol ip, since one of the animals initially included had no positive parasitaemia at 5th dpi and then, it was excluded from the experiment.

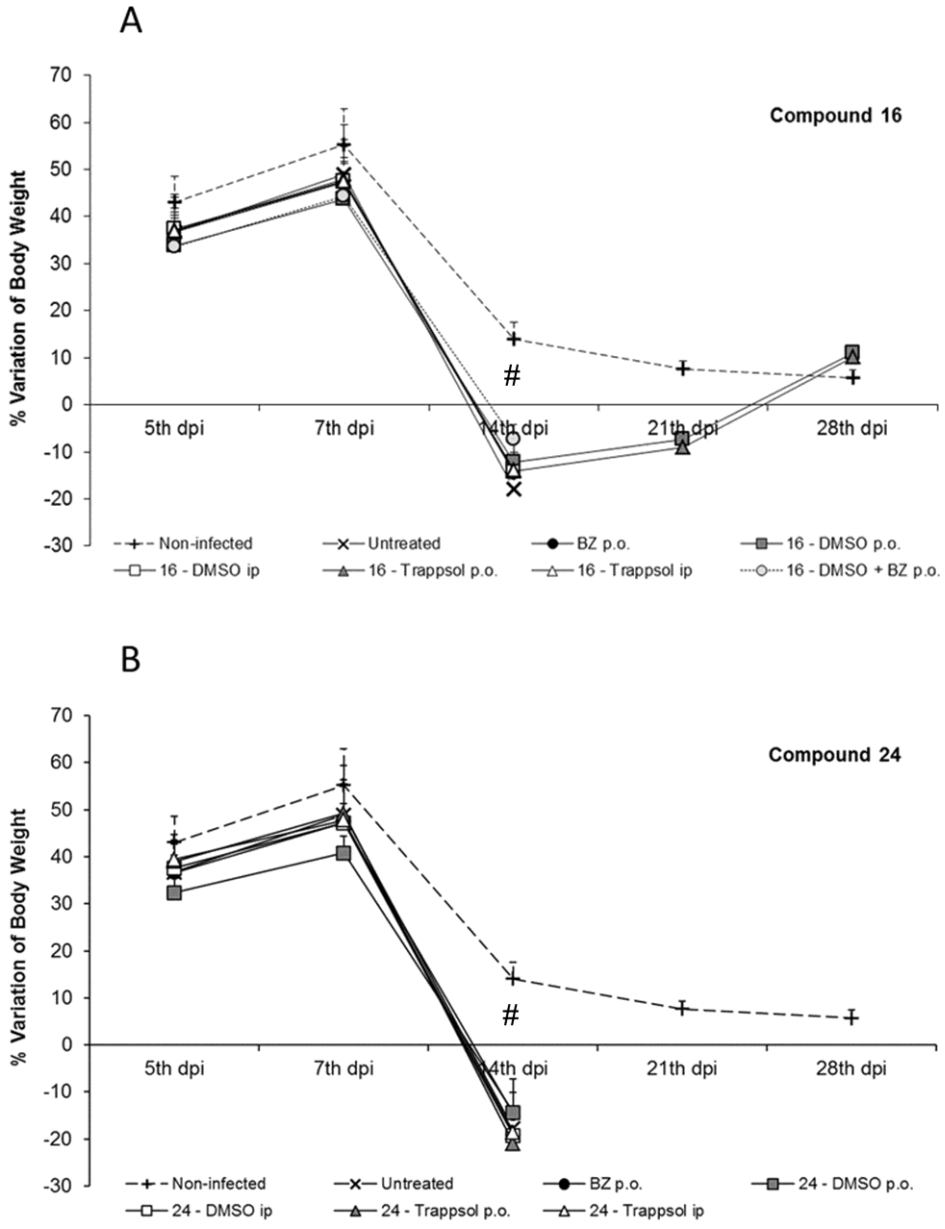

Figure S3. Ponderal curve analysis II

Fig. S3. Ponderal curve analysis of different groups of male Swiss Webster mice (n = 6) treated with five consecutive doses of derivatives 16 and 24, either in monotherapy or co-administered with BZ, vehiculized in 10% DMSO and given p.o. Results are expressed as the mean ± SD. *Significant differences ($P < 0.05$) with the untreated group, one-way ANOVA. #Significant differences ($P < 0.05$) with the group of non-infected mice, one-way ANOVA.