

Library of Selenocyanate and Diselenide Derivatives as In Vivo Antichagasic Compounds Targeting *Trypanosoma Cruzi* Mitochondrion

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Supplementary material

Figure S1. A) Anti-*Trypanosoma cruzi* immunoglobulin G levels. B) Weight percentage of spleens in the chronic Chagas disease.

Figure S2. Nucleic acids levels of *Trypanosoma cruzi*.

Table S1. Activity of benznidazole and compounds tested against cultured epimastigote form of *Trypanosoma cruzi*, toxicity against cultured Vero cells, and selectivity index.

Table S2. Clinical analysis.

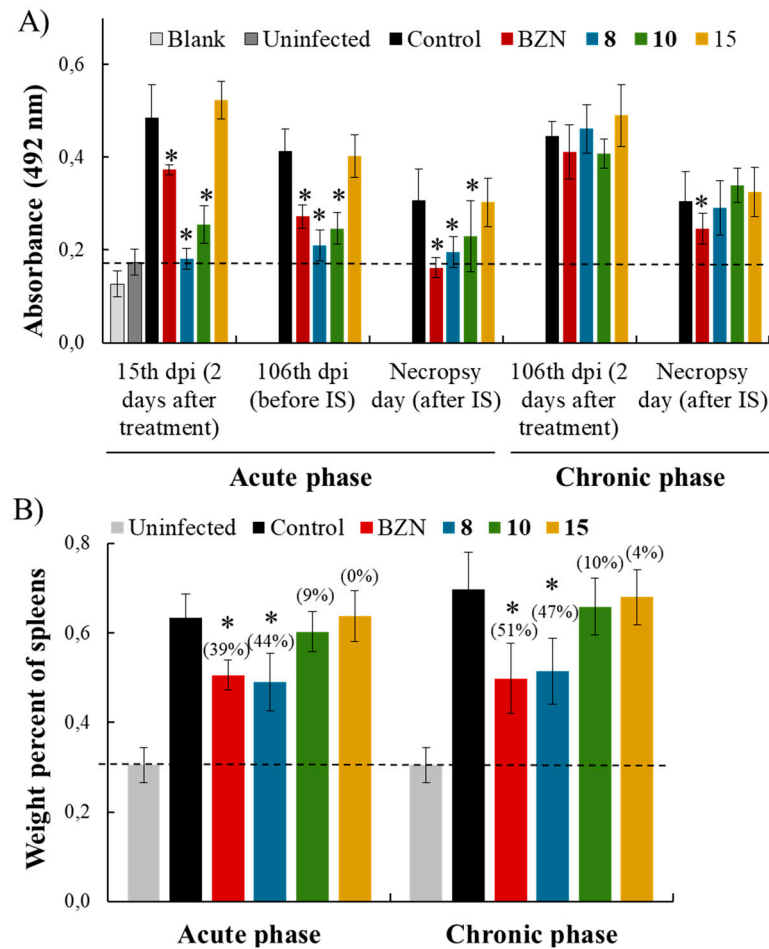


Figure S1. A) Anti-*Trypanosoma cruzi* immunoglobulin G levels, expressed in absorbance at 492 nm, at different days post-infection (dpi) for each group of mice treated during the acute and chronic phases of Chagas disease: control (untreated), benznidazole (BZN), 8, 10 and 15. Blank and uninfected mice are also included. Dashed line shows the cut off for uninfected mice. Values are the means of three mice \pm standard deviation. (IS) immunosuppression. * Significant differences between untreated and treated mice for $\alpha = 0.05$. **B)** Weight percentage of spleens in the chronic Chagas disease for each group of mice treated during the acute and chronic phases of the disease: control (untreated), benznidazole (BZN), 8, 10 and 15. Values are the means of three mice \pm standard deviation. In brackets: reduction of splenomegaly in comparison to the control. * Significant differences between untreated and treated mice for $\alpha = 0.05$.

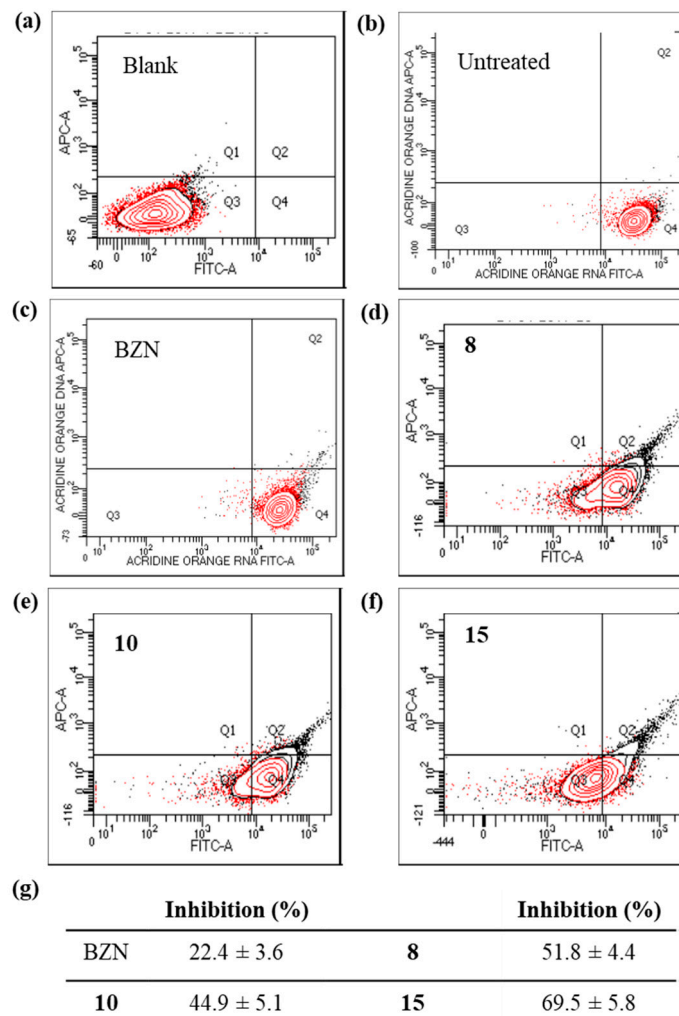


Figure S2. Nucleic acids levels of *Trypanosoma cruzi* exposed to benznidazole (BZN) and compounds at their IC₂₅ concentrations incubated 72 h: (a) blank, (b) untreated (control), (c) BZN, (d) 8, (e) 10, (f) 15. (g) Inhibition, in percentage, in the nucleic acids levels with respect to untreated parasites. Values constitute means of three separate determinations ± standard deviation. Significant differences between untreated and treated parasites for $\alpha = 0.05$.

Table S1. Activity of benznidazole and compounds tested against cultured epimastigote form of *Trypanosoma cruzi*, toxicity against cultured Vero cells, and selectivity index.

Comp	Activity IC50(μM) ^a	Toxicity IC ₅₀ (μM) ^b Vero		SI ^c
		cell		
BZN	16.9 ± 1.8	80.4 ± 7.1		5
1	39.1 ± 2.5	68.4 ± 3.7		2 (0)
2	39.3 ± 4.1	30.7 ± 2.1		1 (0)
3	34.5 ± 2,6	53.3 ± 2.5		1 (0)
4	35.4 ± 3.6	26.8 ± 1.3		1 (0)
5	21.8 ± 2.2	28.6 ± 2.4		1 (0)
6	13.6 ± 0.7	28.2 ± 1.1		2 (0)
7	34.1 ± 2.6	50.5 ± 3.8		1 (0)
8	1.9 ± 0.1	44.3 ± 2.7		23 (5)
9	11.3 ± 0.7	67.3 ± 4.9		6 (1)
10	1.8 ± 0.2	134.6 ± 6.3		75 (15)
11	3.0 ± 0.4	61.3 ± 3.7		20 (4)
12	19.4 ± 1.5	4.9 ± 0.7		0 (0)
13	1.9 ± 0.7	12.8 ± 0.7		7 (1)
14	27.9 ± 2.9	8.9 ± 1.5		0 (0)
15	0.9 ± 0.1	17.9 ± 1.0		20 (4)
16	18.4 ± 2.3	18.9 ± 2.4		1 (0)
17	37.4 ± 2.6	81.2 ± 4.8		2 (0)
18	1.5 ± 0.3	7.3 ± 1.1		5 (1)
19	29.1 ± 3.0	18.5 ± 0.7		0 (0)
20	1.3 ± 0.2	16.4 ± 0.5		13 (3)
21	1.0 ± 0.2	21.7 ± 1.8		22 (4)
22	39.7 ± 2.6	29.8 ± 3.0		0 (0)
23	14.1 ± 0.9	38.9 ± 2.1		3 (1)
24	45.7 ± 2.6	61.8 ± 4.1		1 (0)
25	10.3 ± 0.8	12.6 ± 0.3		1 (0)
26	6.7 ± 0.4	19.6 ± 1.0		3 (1)
27	99.5 ± 6.1	68.3 ± 6.7		0 (0)
28	21.4 ± 0.9	169.2 ± 9.7		8 (2)
29	32.1 ± 1.1	67.4 ± 3.5		2 (0)
30	30.9 ± 1.4	108.6 ± 10.5		4 (1)
31	13.2 ± 0.8	85.5 ± 4.9		6 (1)
32	7.6 ± 0.7	56.5 ± 3.9		7 (1)
33	10.2 ± 0.8	49.7 ± 3.2		5 (1)
34	41.3 ± 2.1	29.8 ± 3.4		0 (0)
35	28.4 ± 1.7	69.0 ± 5.3		2 (0)
36	11.1 ± 0.8	70.8 ± 4.8		6 (1)
37	46.1 ± 3.1	39.6 ± 3.1		0 (0)
38	27.8 ± 1.6	40.3 ± 2.5		1 (0)
39	17.1 ± 1.3	11.7 ± 2.7		0 (0)
40	3.8 ± 0.1	16.0 ± 1.7		4 (1)
41	6.8 ± 0.7	26.5 ± 1.4		4 (1)
42	5.9 ± 0.4	36.0 ± 2.3		6 (1)
43	5.1 ± 0.6	66.9 ± 6.2		13 (3)

44	5.6 ± 1.1	30.8 ± 2.2	6 (1)
45	1.7 ± 0.3	14.3 ± 1.1	8 (2)
46	2.7 ± 0.8	48.6 ± 3.5	18 (4)
47	3.6 ± 0.3	44.7 ± 3.0	12 (2)
48	1.8 ± 0.0	15.7 ± 1.1	9 (2)

^a Inhibition concentration 50 (IC₅₀), concentration (μM) required to inhibit 50% growth, determined using GraphPad Prism 6. ^b Towards Vero cells. ^c Selectivity index (SI), IC₅₀ Vero cells / IC₅₀ epimastigote. Data in brackets refer to the number of times that compounds exceed the reference drug SI. Values are the means of three independent experiments ± standard deviation. BZN, benznidazole.

Table S2. Clinical analysis determined at different days post-infection in groups of mice infected with *Trypanosoma cruzi* and treated with benznidazole and compounds.

	Kidney marker profile		Heart marker profile		Liver marker profile			
	Urea	Uric acid	CK-MB	LDH	AST/GOT	ALT/GPT	Total bilirubin	ALP
	(mg/dL)	(mg/dL)	(U/L)	(U/L)	(U/L)	(U/L)	(mg/dL)	(U/L)
Uninfected mice	38 [32–40]	4.4 [4.0–5.1]	492 [150–630]	3121 [2505–3851]	153 [132–177]	50 [46–62]	0.23 [0.22–0.31]	165 [141–192]
15 th dpi (Control)	31	4.3	535	3275	167	60	0.23	180
15 th dpi and BZN (2 days after treatment)	--	----	=	=	++++	++	++++	=
15 th dpi and 8 (2 days after treatment)	----	----	+	=	=	+++	---	----
Necropsy day of mice (Control)	34	4.0	496	2761	179	49	0.21	161
Necropsy day of mice and BZN	--	-	-	=	+	=	++	=
Necropsy day of mice and 8	-	---	=	=	+	+	-	--

CK-MB, creatine kinase-muscle/brain. LDH, lactate dehydrogenase. AST/GOT, aspartate aminotransferase. ALT/GPT, alanine aminotransferase. ALP, Alkaline phosphatase. BZN, benznidazole. dpi, day post-infection. n= 3, except for uninfected mice (n = 6).

Key: =, variation ≤ 10%; +/-, 10-20% increase/decrease over the range; ++/--, 20-30% increase/decrease over the range; +++/---, 30-40% increase/decrease over the range; ++++/----, > 40% increase/decrease over the range.