

Supplementary Materials: Liposomes Composed by Membrane Lipid Extracts from Macrophage Cell Line as a Delivery of the Trypanocidal *N, N'*-Squaramide 17 towards *Trypanosoma cruzi*

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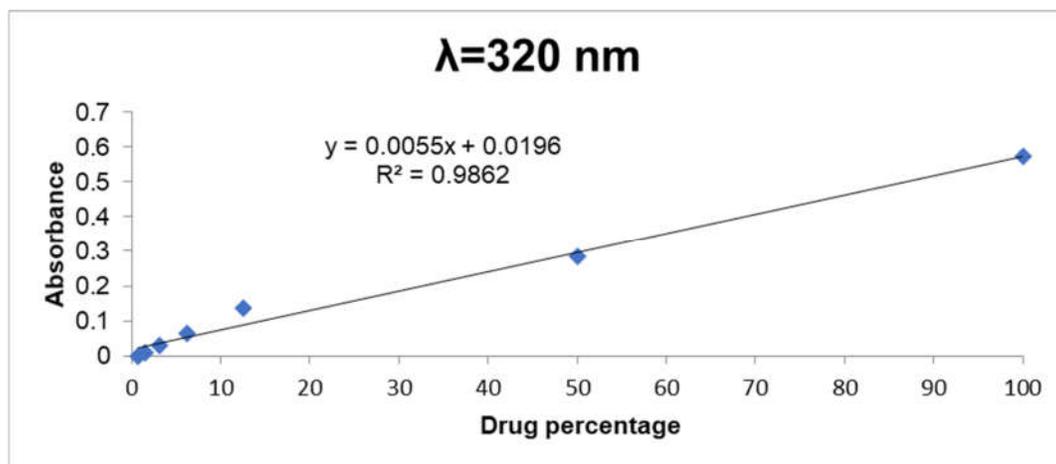


Figure S1. Calibration curve for *N, N'*-Squaramide 17 in different concentrations for percentage.

Table S1. The entrapment efficiency of *N, N'*-Squaramide 17 was measured using a biophotometer by the absorbance of the drug at 320 nm. The experiments were performed in triplicate.

% Entrapment efficiency nanostructures- $\lambda = 320 \text{ nm}$	
1	75.71
2	72.07
3	72.35
Average	73.35
Standard deviation	2.05

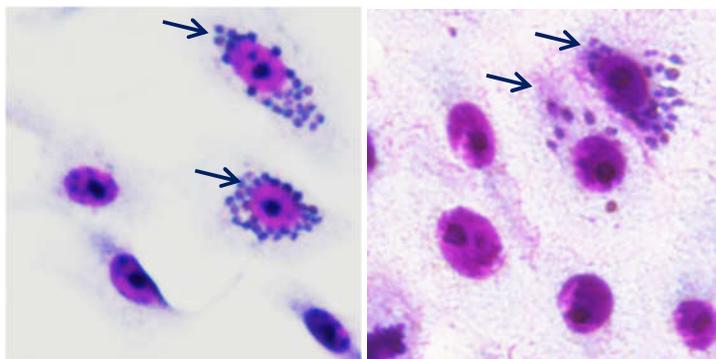


Figure S2. Vero cells infected with *T. cruzi* (arrows). Cells were panoptic-stained.

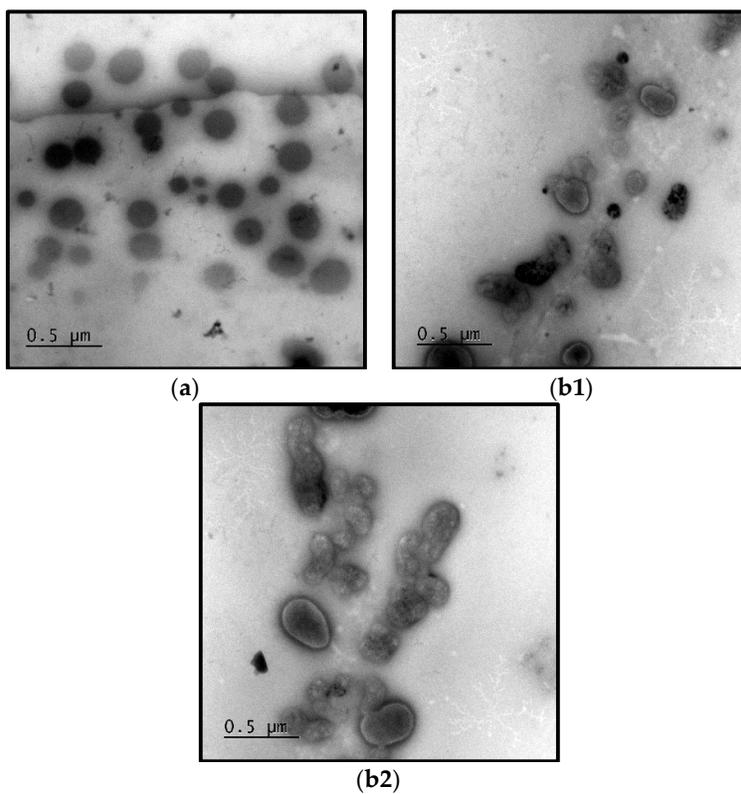
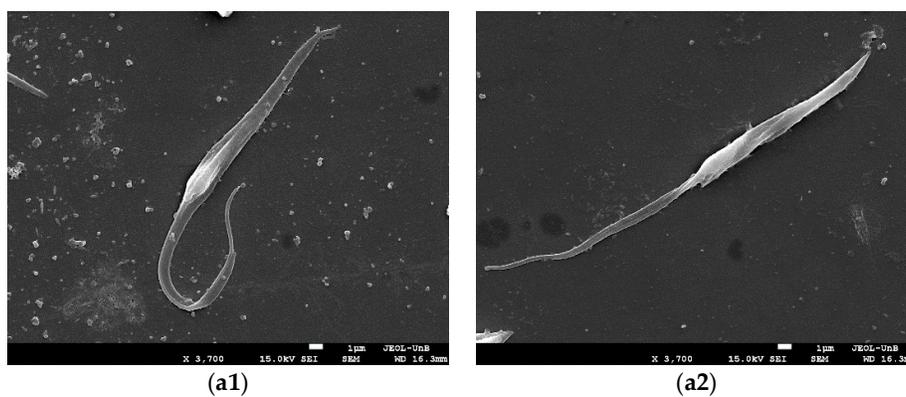


Figure S3. Morphological characteristics of nanostructures analyzed by transmission electron microscopy (TEM). (a) Mimetic lipid membranes containing *N, N'*-Squaramide 17 (MLS) and (b1, b2) Empty mimetic lipid membranes (MLV).



(a1)

(a2)

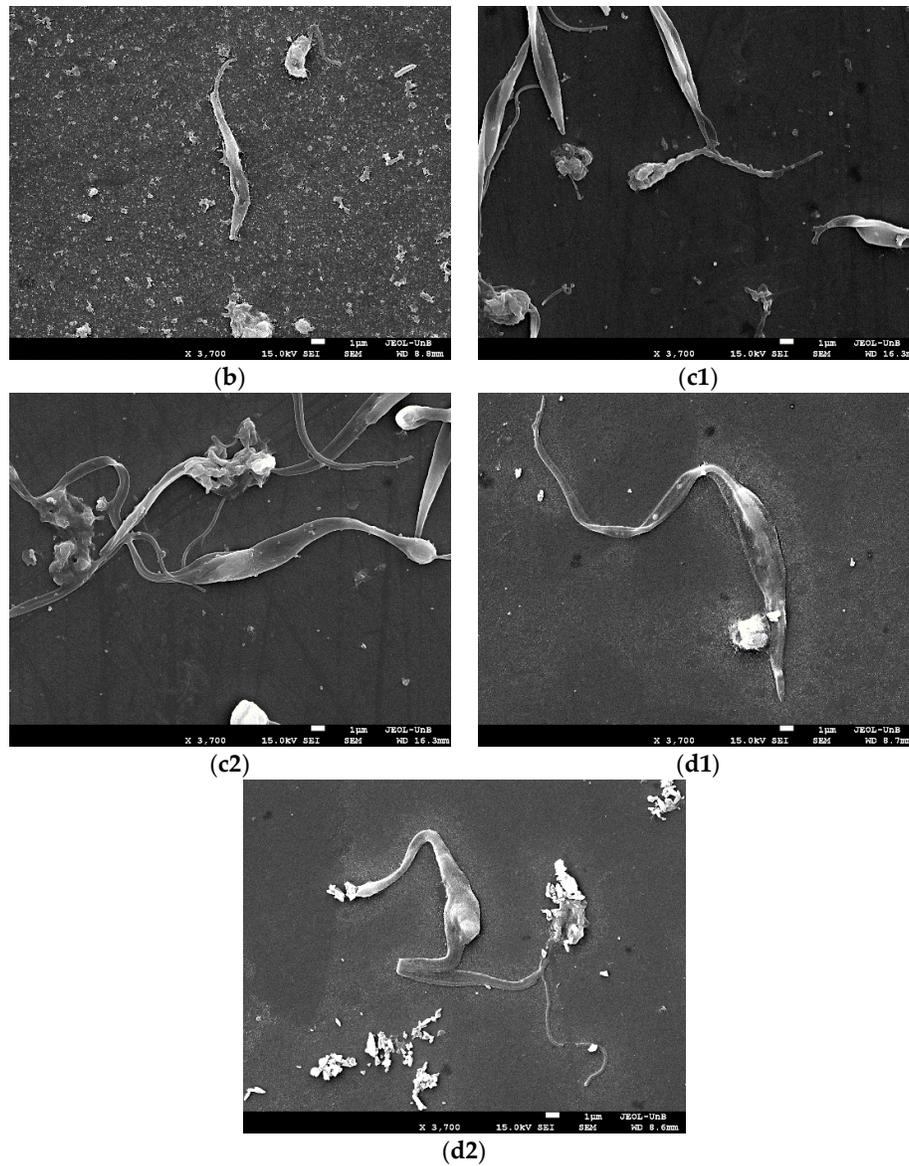


Figure S4. Morphological alterations in *T. cruzi* after compound treatment analyzed by SEM. Epimastigotes treated with Benznidazole at 7.91 μM (BZ); *N, N'*-Squaramide 17 at 6.56 μM (S) and Mimetic lipid membranes containing *N, N'*-Squaramide 17 at 7.93 μM (MLS) for 72 h. (a1, a2) Control (without treatment); (b) BZ; (c1, c2) S; and (d1, d2) MLS.

Table S2. Hydrodynamic diameter and polydispersity index of the different nanostructures evaluated after one and ten days of their production under different pHs.

Nanostructure (Conditions)	Day 1*			Day 10*		
	Hydrodynamic Diameter (nm)	Polydispersity Index (Pdl)	Zeta Potential (mV)	Hydrodynamic Diameter (nm)	Polydispersity Index (Pdl)	Zeta Potential (mV)
MLS	196.2 ± 11.0	0.418 ± 0.086	-61.43 ± 2.30	126.5 ± 0.7	0.292 ± 0.029	-58.0 ± 3.89
MLV	203.1 ± 8.5	0.428 ± 0.092	-12.93 ± 1.21	360.0 ± 87.2	0.424 ± 0.091	-14.4 ± 3.32

*The data refer to the means of three separate experiments (mean ± standard deviation) measured by DLS. Empty Mimetic Lipid Membranes (MLV) and Mimetic Membrane Membranes with *N*, *N'*-Squaramide 17 (MLS).



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