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Supplementary Material

Navigating Drug Repurposing for Chagas Disease: Advances, Challenges, and Opportunities

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Supporting Table 1. Summary of selected reported examples of drug repurposing for CD, including their current uses and biological activities against the *T. cruzi* parasite. References: The Selectivity Index (SI) is defined as the ratio of the toxic concentration of the compound in mammal cells against its effective bioactive concentration in amastigotes of *T. cruzi*. AMA: amastigotes; EPI: epimastigotes; T: trypomastigotes; BT: bloodstream trypomastigotes.

Drug	Reference	Original function	Biological Activity against *T. cruzi* (EC_{50})	Other features
1 Benznidazole	Revollo et al., 2019 [1]	Medication used in the treatment of Chagas disease	4.00 μM (AMA) 4.02 μM (EPI) 5.73 μM (T)	In use since 1971
2 Nifurtimox	Revollo et al., 2019 [1]	Medication used in the treatment of Chagas disease	2.61 μM (AMA) 2.46 μM (EPI) 3.60 μM (T)	Approved in 1965
3 Camptothecin	Sykes and Avery, 2015	Topoisomerase I inhibitor	90 nM (AMA) SI > 56	FDA-Approved drug library

4 Clemastine	Sykes and Avery, 2015 De Rycker, 2016	First-generation H1 histamine antagonist	0.29 μM (AMA) SI > 63 (Sykes) 0.1 μM (AMA) SI = 251 (De Rycker)	NIH and SelleckChem FDA-approved drug library
5 Crystal violet	Sykes and Avery, 2015 Sayé et al., 2020	Inhibitor of proline uptake through the proline permease TcAAAP069	90 nM (AMA) SI > 320 0.84 μM (T) (Sykes) 0.3 μM (AMA) 0.3 μM (BT) 12.7 μM (EPI) (Sayé)	Inhibitor of proline transport (IC_{50} of 7.1μM). Synergistic effect in combination with BZN
6 Clotrimazole	Sykes and Avery, 2015 Kaiser et al., 2015	Antifungal	170 nM (AMA) SI > 107 (Sykes) 6 nM (AMA) SI = 498 (Kaiser)	Inhibitor of the 14-alpha-sterol demethylase
7 Tadalafil	Kaiser et al., 2015	Phosphodiesterase type 5 inhibitor	8.60 μM (AMA) SI = 26	cGMP-specific 3'-5'-cyclic phosphodiesterase inhibitor
8 Mebeverine	Kaiser et al., 2015	Antispasmodic	3.89 μM (AMA) SI = 18	Serotonine 5-HT3 receptor agonist
9 Nilotinib	Juárez-Saldivar et al., 2020	Tyrosine kinase inhibitor (TKI)	6 μM (EPI)	*Tc*DHFR-TS inhibitor
10 Glipizide	Juárez-Saldivar et al., 2020	Anti-type 2 diabetes mellitus	13.4 μM (EPI)	*Tc*DHFR-TS inhibitor
11 Glyburide	Juárez-Saldivar et al., 2020	Anti-type 2 diabetes mellitus	66 μM (EPI)	*Tc*DHFR-TS inhibitor
12 Gliquidone	Juárez-Saldivar et al., 2020	Anti-type 2 diabetes mellitus	12 μM (EPI)	*Tc*DHFR-TS inhibitor
13 Etidronate	Valera-Vera et al., 2020	Treatment of Paget's disease	No data in this study	Potential *Tc*ENO inhibitor (by docking)

14 GLK2-003	Mercaldi et al., 2019	TimTec Library	2.1 μM (AMA) SI = 38.4	Inhibitor of *T. cruzi* glucokinase (IC_{50} = 6.1 μM)
15 GLK2-004	Mercaldi et al., 2019	TimTec Library	2.9 μM (AMA) SI > 36	Inhibitor of *T. cruzi* glucokinase (IC_{50} = 4.8 μM)
16 NEQ176	Wiggers et al., 2013	Molecule of the ZINC Database	108 μM (T) SI > 2.5 (T)	Cruzain inhibitor with an IC_{50} of 68.5 μM
17 NEQ42	Wiggers et al., 2013	Molecule of the ZINC Database	10.6 μM (T) SI = 5 (T)	Cruzain inhibitor with an IC_{50} of 21.6 μM
18 Levothyroxine	Sayé et al., 2020	Synthetic form of the thyroid hormone thyroxine T4	121 μM (EPI)	Cruzipain inhibitor with an IC_{50} of 38.43 ± 6.82 μM
19 Disulfiram	Almeida-Silva et al., 2022	Drug to treat chronic alcoholism	Pro-drug of diethyl-dithiocarbamate **20**	In clinical trial in combination with BZN
20 Diethyl-dithiocarbamate	Almeida-Silva et al., 2022	A major metabolite of disulfiram	1.48 μM (EPI)	Synergistic effect combined with BZN (increased the selectivity 13-fold)
21 Imatinib	Simões-Silva et al., 2019a	Tyrosine kinase inhibitor (TKI)	24.8 μM (AMA) SI = 1.5 30.0 μM (BT)	IMB + BZ in fixed-ratio proportions was additive
22 LS2/89	Nesic de Freitas et al., 2023	Synthetic analogue of Imatinib	0.19 μM (AMA) SI > 60 2.67 μM (BT)	>10-times better activities than imatinib
23 NSC-706744	Bernatchez et al., 2020	DNA topoisomerase inhibitors	0.44 nM (AMA) SI = 214	Molecule of ReFRAME library
24 ASP-8273	Bernatchez et al., 2020	EGFR inhibitor	2.7 nM (AMA) SI = 191	Molecule of ReFRAME library
25 Miltefosine	Gulin et al., 2022	Anti-leishmanial	0.51 μM (AMA) SI = 112 31.2 μM (BT)	MLT+BZ *in vitro* synergistic effect on trypomastigotes and an additive on amastigote

26 Tamoxifen	Miguel et al., 2010	Anti-breast cancer	2.7 μM (AMA) 0.7 μM (T) 12.3 μM (EPI)	No differences in parasitemia and mortality in models of acute CD
27 Azelastine	De Rycker, 2016	H_1 receptor-blocking (antihistaminic)	0.25 μM (AMA) SI = 63	NIH and SelleckChem FDA-approved drug library
28 Loratadine	Sayé et al., 2020	Anti-histaminic	13.2 μM (AMA) SI = 5.5 12.9 μM (BT) 26.4 μM (EPI)	Inhibitor of proline transport (IC_{50} of 23.1μM). Synergistic effect in combination with BZN
29 Cyproheptadine	Sayé et al., 2020	Anti-histaminic	10.7 μM (AMA) SI = 9.1 11.3 μM (BT) 52.6 μM (EPI)	Inhibitor of proline transport (IC_{50} of 71.9μM). Synergistic effect in combination with BZN
30 Cinnarizine	Alberca et al., 2018	Anti-histaminic and calcium channel blocker	6.05 μM (EPI)	Effect on putrescine uptake (52% of initial velocity reduction)
31 Ifenprodil	De Rycker, 2016	Inhibitor of the NMDA receptor	0.50 μM (AMA) SI = 16	NIH and SelleckChem FDA-approved drug library
32 Ziprasidone	De Rycker, 2016	Atypical antipsychotic	1.58 μM (AMA) SI = 100	NIH and SelleckChem FDA-approved drug library
33 Sertraline	Ferreira et al., 2018	Antidepressant of the selective serotonin reuptake inhibitor (SSRI) class	1.4 μM (AMA) SI=17.4 (AMA) 14 μM (BT) 1.8 μM (T)	Depletion of ATP levels. Affected the parasite TcIDH2 (by in silico).
34 Promazine	Reigada et al., 2019a	First generation antipsychotic	3.8 μM (AMA) 3.4 μM (BT) 34.7 μM (EPI)	Inhibitor of *Tc*PAT12 (by docking)
35 Chlorpromazine	Reigada et al., 2019a	Antipsychotic	1.9 μM (AMA) 2.7 μM (BT) 41.4 μM (EPI)	Inhibitor of *Tc*PAT12 (by docking)

36 Clomipramine	Reigada et al., 2019a	Antidepressant	2.9 μM (AMA) 1.3 μM (BT) 39.7 μM (EPI)	Inhibitor of *Tc*PAT12 (by docking) Synergistic effect combined with BZN
37 Naproxen	Adasme et al., 2020	Nonsteroidal anti-inflammatory	58.5 μM (BT) >400 μM (EPI)	Inhibition of parasitemia of 85.8% at 8 hours of treatment (*in vivo*)
38 Nimesulide	Trindade et al., 2021	Non-selective non-steroidal anti-inflammatory drug (NSAID)	12.93 μM (EPI)	Significant changes in cell organelles (ultrastructure). Affected cell redox balance.
39 Atorvastatin	Araujo-Lima et al., 2018	Statin medication	7.3 μM (AMA) SI = 21 (AMA) 7.1 μM (BT) SI = 51 (BT)	Synergistic interactions with BZN against in both trypomastigotes and intracellular forms
40 Clofibrate	De Rycker, 2016	Lipid-lowering agent	6.31 μM (AMA) SI = 2.5	NIH and SelleckChem FDA-approved drug library
41 Amiodarone	Barbosa et al., 2022	Antiarrhythmic	0.51 μM (AMA)	BZN+AMD attenuated the infection-triggered cytoskeleton damage of host cells
42 Carvedilol	Rivero et al., 2021	Beta-blocker	100% growth inhibition at 10 μM (EPI)	Diminishes the peak of whole-body parasite burden in infected mice
43 Manidipine	Correa et al., 2021	Calcium channel blocker (antihypertensive)	0.1 μM (AMA) SI > 1459 3 μM (T)	Decreased ATP levels
44 Benidipine	Bellera et al., 2015	Calcium channel blocker	19.5 μM (EPI)	Lower parasitemia at doses much smaller than the one used for the positive control BZN
45 Terconazole	Reigada et al., 2019b	Antifungal	5.9 μM (AMA) 4.6 μM (T) 25.7 μM (EPI)	Inhibited *T. cruzi* cytochrome P450 14-alpha-demethylase (by docking)
46 Bifonazole	Kaiser et al., 2015	Antifungal	3 nM (AMA) SI > 1000	Inhibitor of the 14-alpha-sterol demethylase

47 Econazole	Kaiser et al., 2015	Antifungal	40 nM (AMA) SI = 390	Inhibitor of the 14-alpha-sterol demethylase
48 Miconazole	Kaiser et al., 2015	Antifungal	4 nM (AMA) SI = 383	Inhibitor of the 14-alpha-sterol demethylase
49 Tioconazole	Kaiser et al., 2015	Antifungal	64 nM (AMA) SI = 304	Inhibitor of the 14-alpha-sterol demethylase
50 Itraconazole	Kaiser et al., 2015	Antifungal	4 nM (AMA) SI = 278	Inhibitor of the 14-alpha-sterol demethylase
51 Ketoconazole	Kaiser et al., 2015	Antifungal	0.27 μM (AMA) SI = 189	Inhibitor of the 14-alpha-sterol demethylase
52 Posaconazole	Rocha-Hasler et al., 2021	Antifungal	2 nM (AMA) SI > 700	Failed in clinical trials for CD
53 E1224	Machado et al., 2020	Antifungal	1.84 nM (AMA) SI > 200 79.5 nM (T)	Failed in clinical trials for CD
54 Metronidazole	Simões-Silva et al., 2017	Antibiotic and antiprotozoal	>200 μM (AMA) >200 μM (BT)	Synergistic effect in combination with BZN and prevented mortality (70%)
55 Ciprofloxacin	Adasme et al., 2020	Fluoroquinolone antibiotic	21.3 μM (BT) >400 μM (EPI)	Inhibition of parasitemia of 66.7% at 8 hours of treatment (*in vivo*)
56 Piperacillin	Palos et al., 2017	β-lactam antiobiotic	30.6 μM (AMA)	A short-term *in vivo* evaluation showed a reduction of parasitemia in infected mice
57 Clofazimine	Bellera et al., 2015 Sayé et al., 2020	Bacteriostatic anti-leprosy	10.6 μM (EPI) (Bellera) 1.1 μM (AMA) SI = 36 2.8 μM (BT) 9.3 μM (EPI) (Sayé)	Lower parasitemia at doses much smaller than the one used for the positive control BZN. Inhibitor of proline transport (IC_{50} of 4.3μM). Synergistic effect in combination with BZN.

58 Saquinavir	Bellera et al., 2015	Protease inhibitors (antiretroviral)	100% growth inhibition at 20 μM (EPI)	Solubility problem
59 348U87	Bernatchez et al., 2020	Antiherpetic	0.63 nM (AMA) SI = 1294	Molecule of ReFRAME library
60 MMV687776	Duffy et al., 2017	Anti-lymphatic filariasis	2.39 μM (AMA)	Molecule of the Pathogen Box
61 MMV637229	Duffy et al., 2017	H1 histamine antagonist	1.22 μM (AMA)	Molecule of the Pathogen Box
62 MMV689028	Duffy et al., 2017	Originated from the GSK	0.67 μM (AMA)	Molecule of the Pathogen Box
63 MMV689029	Duffy et al., 2017	Not informed	2.70 μM (AMA)	Molecule of the Pathogen Box
64 MMV688796	Duffy et al., 2017	Not informed	2.07 μM (AMA)	Molecule of the Pathogen Box
65 MMV688371	Duffy et al., 2017	Not informed	0.62 μM (AMA)	Molecule of the Pathogen Box
66 MMV689709	Duffy et al., 2017	Not informed	3.32 μM (AMA)	Molecule of the Pathogen Box
67 Ivermectin	Fraccaroli et al., 2022	Broad-spectrum antiparasitic	0.3 μM (AMA) SI > 12 10.4 μM (T) 5.3 μM (EPI)	IVM + BZ in fixed-ratio proportions was additive
68 Chloroquine	Pandey et al., 2022	Antimalarial	0.4 μM (AMA)	Synergistic effect combined with BZN (2 drugs combination) and with cochicine (3 drugs)
69 Levamisole	Simões-Silva et al., 2019b	Antihelminthic	No data in this study	Parasitaemia suppression was achieved in combination with BZN
70 Resveratrol	Rodriguez et al., 2022	Activator of KDACs type III and antioxidant	50.3 μM (T) (Campo, 2017)	Reduced the percentage of infected cells by 50-70% (protective effect)

71 Fexinidazole	Bahia et al., 2012	Medication used to treat African trypanosomiasis	1.1 μM (AMA) SI > 100	Phase II clinical trial was completed at the end of 2022
72 Isotretinoin	Reigada et al., 2017	Acne medicine	130 nM (T) SI = 920 (T) 30.6 μM (EPI)	Inhibitor of the polyamine transport (TcAAAP). IC_{50} = [4.6–10.3] μM.
73 Amlodipine	Machado et al., 2020	Calcium channel blocker	2.75 μM (AMA) SI = 33.2 (AMA) 4.9 μM (T)	Amlodipine + posaconazole, synergistic effect in mice

References:

[1] Revollo, S., Oury, B., Vela, A., Tibayrenc, M., Sereno, D. (2019). In Vitro Benznidazole and Nifurtimox Susceptibility Profile of Trypanosoma cruzi Strains Belonging to Discrete Typing Units TcI, TcII, and TcV. Pathogens. 8(4):197. doi: 10.3390/pathogens8040197

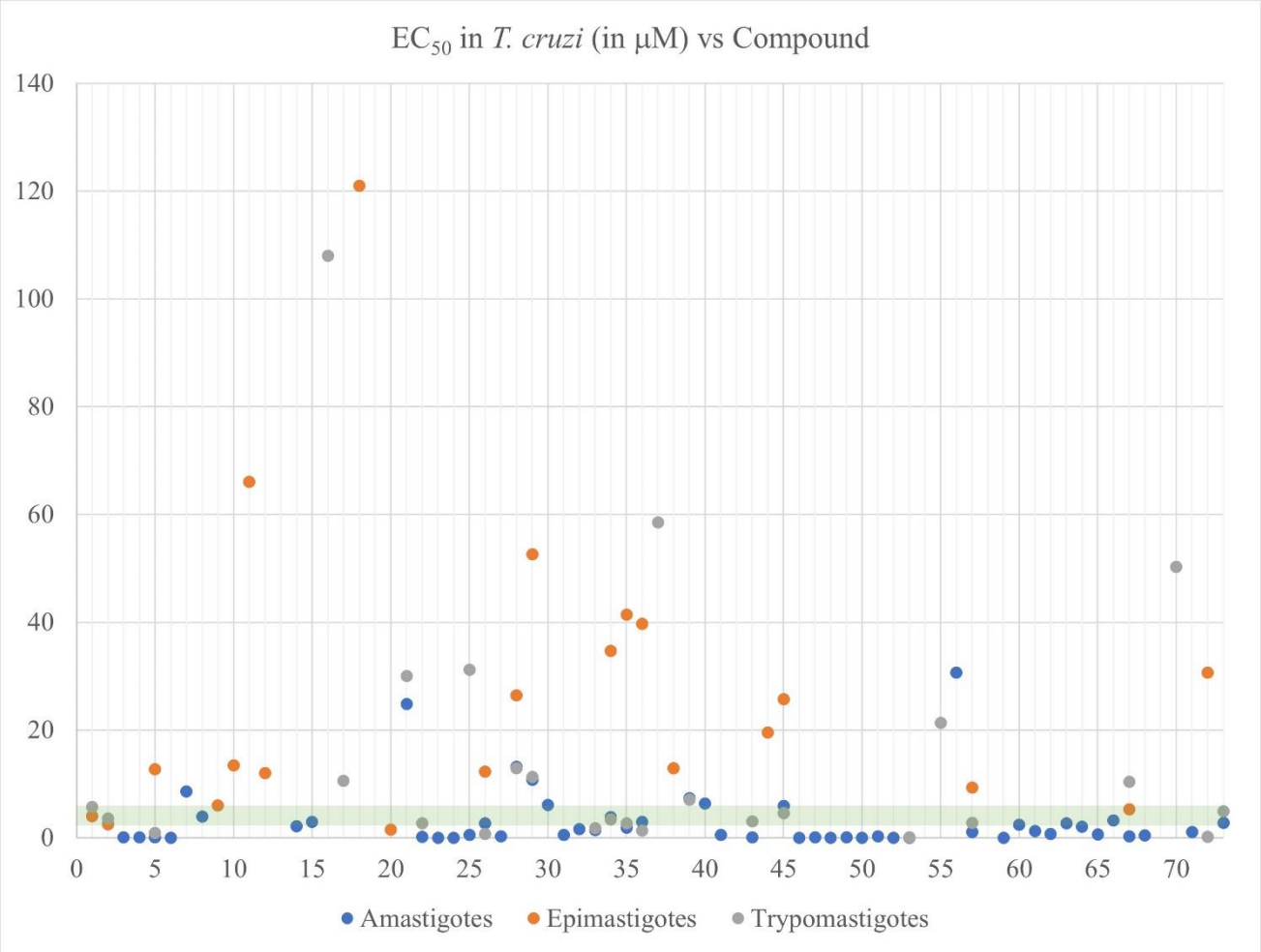

Figure S1. Comparative analysis of the *in vitro* activity of compounds against different stages of *T. cruzi*, quantified by EC_{50} (in μM). Within the green area, lies the range of concentrations where the current chemotherapy agents (Benznidazole **1** and Nifurtimox **2**) are effective *in vitro* against different stages of *T. cruzi*.

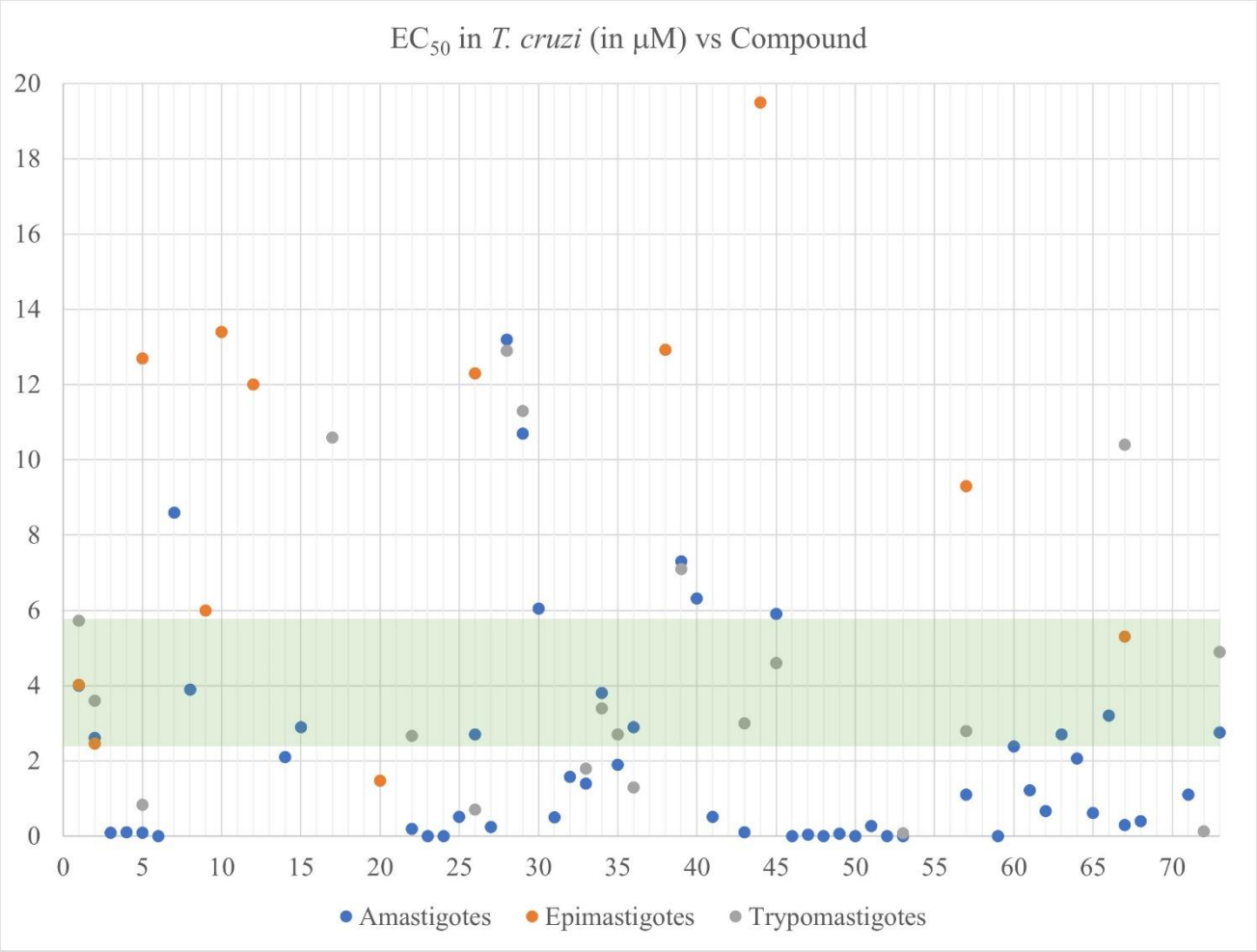

Figure S2. Comparative analysis of the subset of compounds with EC_{50} < 20 μM *in vitro* activities against *T. cruzi* amastigotes, quantified by EC_{50} (in μM). Within the green area, lies the range of concentrations where the current chemotherapy agents (Benznidazole **1** and Nifurtimox **2**) are effective *in vitro* against different stages of *T. cruzi*.

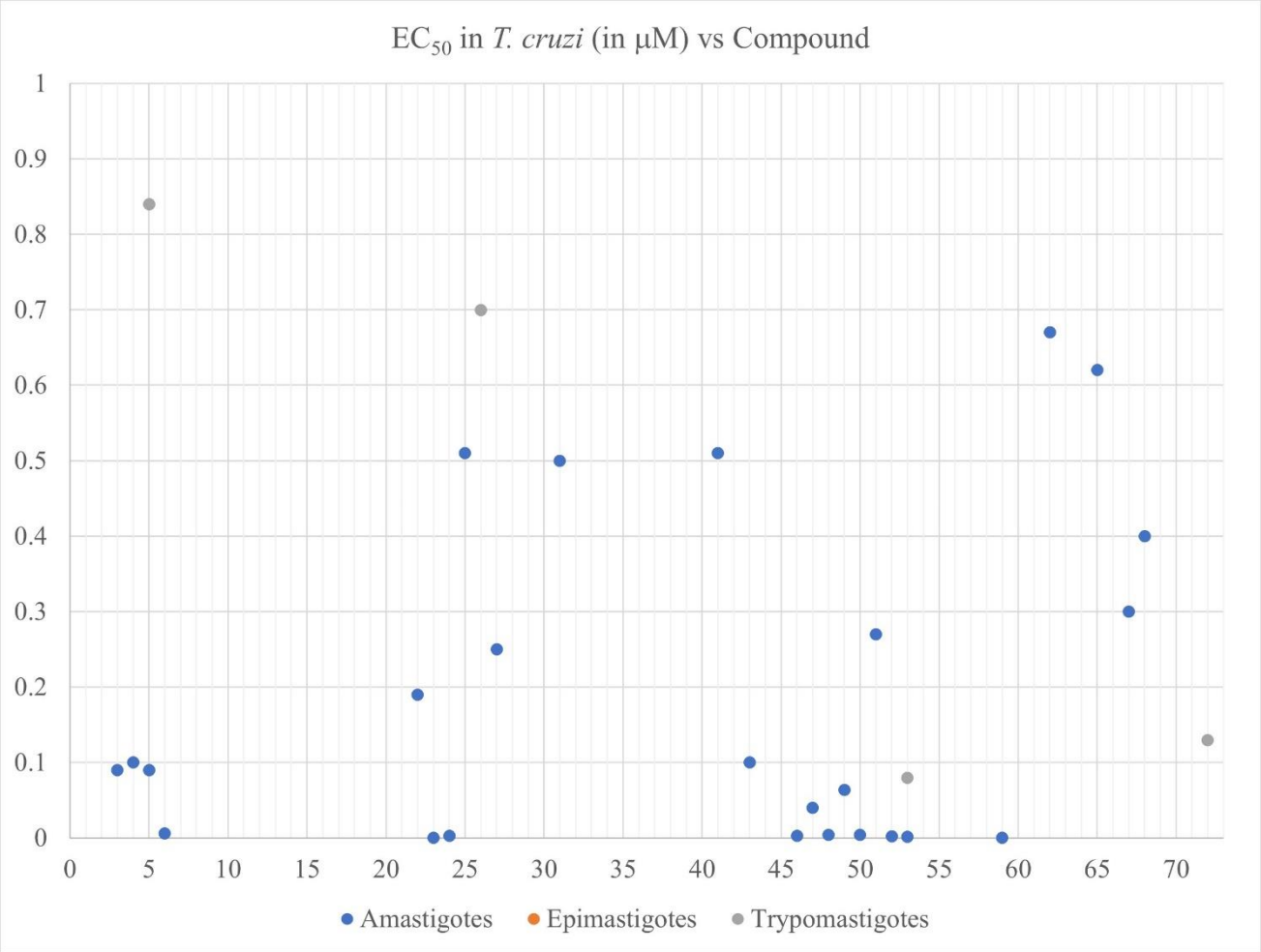

Figure S3. Comparative analysis of the subset of compounds with EC_{50} < 1 μM (submicromolar and nanomolar) *in vitro* activities against *T. cruzi* amastigotes, quantified by EC_{50} (in μM).