

Nitroheterocyclic compounds are more efficacious than CYP51 inhibitors against *Trypanosoma cruzi*: implications for Chagas disease drug discovery and development

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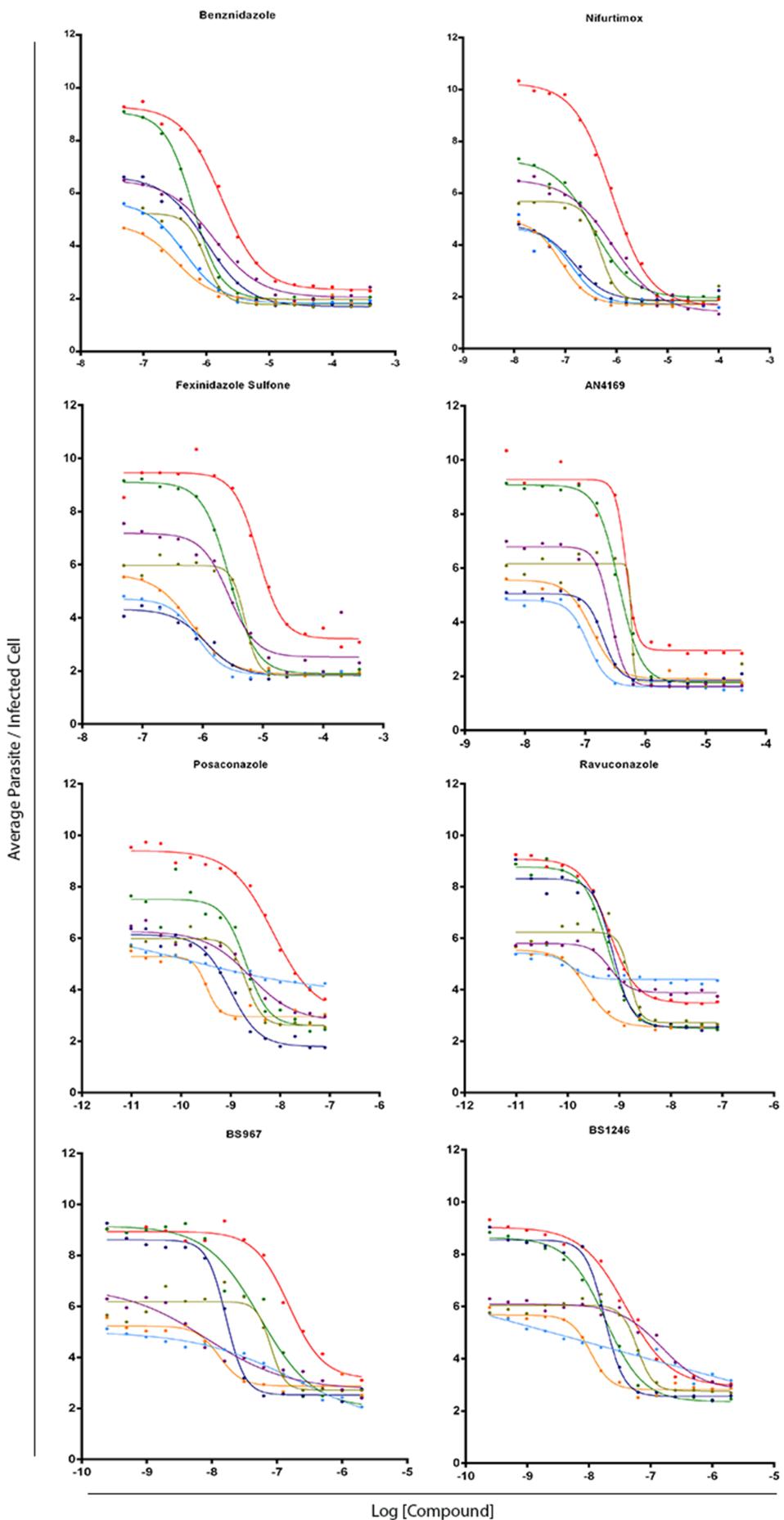
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Supplementary Table S1: Details of the *Trypanosoma cruzi* used in this study

T. cruzi	DTU	Original host¹⁻⁴	Geographical origin^{2,4-9}	Genotyping Methods
Dm28c	I	Opossum <i>Didelphis marsupialis</i>	Carabobo, Venezuela	<i>TcSC5D</i> single locus amplification and sequencing ¹⁰⁻¹⁸
	Y	Human	São Paulo, Brazil	<i>TcSC5D</i> single locus amplification and sequencing ^{11,19-21}
ARMA13 cl1	III	Armadillo <i>Dasypus novemcinctus</i>	Campo Lorro, Paraguay	Multilocus microsatellite genotyping ^{4,22} Large Subunit rDNA PCR product size polymorphism & PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) assay ^{23,24} Multilocus Sequence Typing (MLST) ²⁵
ERA cl2	IV	Human	Anzoátegui, Venezuela	Multilocus microsatellite genotyping ⁴ Large Subunit rDNA PCR product size polymorphism & PCR-restriction fragment length polymorphism (PCR-RFLP) assay ²⁴
92-80 cl2	V	Human	Santa Cruz, Bolivia	Multilocus microsatellite genotyping ⁴ Large Subunit rDNA PCR product size polymorphism & PCR-restriction fragment length polymorphism (PCR-RFLP) assay ²⁴ Multilocus sequence typing (MLST) ²⁵
CL Brener	VI	<i>Triatoma infestans</i>	Rio Grande do Sul, Brazil	rRNA & miniexon gene sequence ²⁶ Multilocus microsatellite genotyping ⁴ Large Subunit rDNA PCR product size polymorphism & PCR-restriction fragment length polymorphism (PCR-RFLP) assay ²⁴ Multilocus sequence typing (MLST) ²⁵ <i>TcSC5D</i> single locus amplification and sequencing ¹¹
Tulahuen	VI	Human	Tulahuen, Chile	rRNA& miniexon gene sequence ²⁶



(Previous page) Supplementary Figure S1. Compound activity measurements based on raw data of the average number of parasites per infected cell. Experimental conditions and datasets are the same of manuscript's Figure 1. Each dose-response curve represents one strain or clone as follows: Dm28c, purple; Y, red; ARMA13 cl1, orange; ERA cl2, light green; 92-80 cl2, light blue; CL Brener, dark blue; and Tulahuen, dark green. The X-axis shows log of compound molar concentrations (M) and Y-axis shows the non-normalized activity (raw data) based on the measurement average number of parasites per infected cell. Data refers to mean values of at least two independent experiments.

References

1. Marin-Neto, J. A. *et al.* Rationale and design of a randomized placebo-controlled trial assessing the effects of etiologic treatment in Chagas' cardiomyopathy: the BENznidazole Evaluation For Interrupting Trypanosomiasis (BENEFIT). *Am Heart J* **156**, 37–43 (2008).
2. Zingales, B. *et al.* A new consensus for *Trypanosoma cruzi* intraspecific nomenclature: second revision meeting recommends TcI to TcVI. in *Mem. Inst. Oswaldo Cruz* **104**, 1051–1054 (2009).
3. Marin-Neto, J. A. *et al.* The BENEFIT trial: testing the hypothesis that trypanocidal therapy is beneficial for patients with chronic Chagas heart disease. *Mem. Inst. Oswaldo Cruz* **104 Suppl 1**, 319–324 (2009).
4. Lewis, M. D. *et al.* Flow cytometric analysis and microsatellite genotyping reveal extensive DNA content variation in *Trypanosoma cruzi* populations and expose contrasts between natural and experimental hybrids. *Int. J. Parasitol.* **39**, 1305–1317 (2009).
5. Castro, J. A., de Mecca, M. M. & Bartel, L. C. Toxic side effects of drugs used to treat Chagas' disease (American trypanosomiasis). *Hum Exp Toxicol* **25**, 471–479 (2006).
6. Viotti, R. *et al.* Side effects of benznidazole as treatment in chronic Chagas disease: fears and realities. *Expert Rev Anti Infect Ther* **7**, 157–163 (2009).
7. Yun, O. *et al.* Feasibility, drug safety, and effectiveness of etiological treatment programs for Chagas disease in Honduras, Guatemala, and Bolivia: 10-year experience of Médecins Sans Frontières. *PLoS Negl. Trop. Dis.* **3**, e488 (2009).
8. Sosa Estani, S. *et al.* Efficacy of chemotherapy with benznidazole in children in the indeterminate phase of Chagas' disease. *Am. J. Trop. Med. Hyg.* **59**, 526–529 (1998).
9. Sosa-Estani, S., Colantonio, L. & Segura, E. L. Therapy of Chagas disease: implications for levels of prevention. *J. Trop. Med.* **2012**, (2012).
10. Urbina, J. A. *et al.* Antiproliferative effects and mechanism of action of SCH 56592 against *Trypanosoma (Schizotrypanum) cruzi*: *in vitro* and *in vivo* studies. *Antimicrob. Agents. Chemother.* **42**, 1771–1777 (1998).
11. Cosentino, R. O. & Aguero, F. A simple strain typing assay for *Trypanosoma cruzi*: discrimination of major evolutionary lineages from a single amplification product. *PLoS Negl. Trop. Dis.* **6**, e1777 (2012).
12. Molina, J. *et al.* Activities of the triazole derivative SCH 56592 (posaconazole) against drug-resistant strains of the protozoan parasite *Trypanosoma (Schizotrypanum) cruzi* in immunocompetent and immunosuppressed murine hosts. *Antimicrob. Agents. Chemother.* **44**, 150–155 (2000).
13. Urbina, J. A., Payares, G., Sanoja, C., Lira, R. & Romanha, A. J. *In vitro* and *in vivo* activities of rauconazole on *Trypanosoma cruzi*, the causative agent of Chagas disease. *Int. J. Antimicrob. Agents* **21**, 27–38 (2003).
14. Ferraz, M. L., Gazzinelli, R. T., Alves, R. O., Urbina, J. A. & Romanha, A. J. The Anti-*Trypanosoma cruzi* activity of posaconazole in a murine model of acute Chagas' disease is less dependent on gamma interferon than that of benznidazole. *Antimicrob. Agents. Chemother.* **51**, 1359–1364 (2007).
15. Olivieri, B. P. *et al.* A comparative study of posaconazole and benznidazole in the prevention of heart damage and promotion of trypanocidal immune response in a murine model of Chagas disease. *Int. J. Antimicrob. Agents* **36**, 79–83 (2010).
16. Diniz, L. de F. *et al.* Effects of rauconazole treatment on parasite load and immune response in dogs experimentally infected with *Trypanosoma cruzi*. *Antimicrob. Agents. Chemother.* **54**, 2979–2986 (2010).
17. Veiga-Santos, P. *et al.* Effects of amiodarone and posaconazole on the growth and ultrastructure of *Trypanosoma cruzi*. *Int. J. Antimicrob. Agents* **40**, 61–71 (2012).
18. Buckner, F. S. Experimental chemotherapy and approaches to drug discovery for *Trypanosoma cruzi* infection. *Adv. Parasitol.* **75**, 89 (2011).
19. Merck. STOPCHAGAS: A Study of the Use of Oral Posaconazole (POS) in the Treatment of Asymptomatic Chronic Chagas Disease. (ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US)). at

- <<http://clinicaltrials.gov/ct2/show/NCT01377480?term=A+Study+of+the+Use+of+Oral+Posaconazole%28POS%29+in+the+Treatment+of+Asymptomatic+Chronic+Chagas+Disease&rank=1>>
- 20. Diseases, D. F. N. *Proof of Concept Study of E1224 to Treat Adults Patients with Chagas Disease.* (ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US)). at <<http://clinicaltrials.gov/ct2/show/NCT01489228?term=chagas+disease&rank=9>>
 - 21. Hospital Universitari Vall d'Hebron Research Institute. *Clinical Trial For The Treatment Of Chronic Chagas Disease With Posaconazole And Benznidazole (CHAGASAZOL).* (ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US)). at <<http://clinicaltrials.gov/ct2/show/study/NCT01162967?term=chagasazol&rank=1>>
 - 22. Bahia, M. T. *et al.* Fexinidazole: A Potential New Drug Candidate for Chagas Disease. *PLoS Negl. Trop. Dis.* **6**, e1870 (2012).
 - 23. Bustamante, J. M., Craft, J. M., Crowe, B. D., Ketchie, S. A. & Tarleton, R. L. New, combined, and reduced dosing treatment protocols cure *Trypanosoma cruzi* infection in Mice. *J. Infect. Dis.* (2013). doi:10.1093/infdis/jit420
 - 24. Lewis, M. D. *et al.* Genotyping of *Trypanosoma cruzi*: systematic selection of assays allowing rapid and accurate discrimination of all known lineages. *Am. J. Trop. Med. Hyg.* **81**, 1041–1049 (2009).
 - 25. Yeo, M. *et al.* Multilocus sequence typing (MLST) for lineage assignment and high resolution diversity studies in *Trypanosoma cruzi*. *PLoS Negl. Trop. Dis.* **5**, e1049 (2011).
 - 26. Brisse, S., Verhoef, J. & Tibayrenc, M. Characterisation of large and small subunit rRNA and mini-exon genes further supports the distinction of six *Trypanosoma cruzi* lineages. *Int. J. Parasitol.* **31**, 1218–1226 (2001).