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Statins do not reduce the parasite burden during experimental *Trypanosoma cruzi* infection

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ABSTRACT Cardiomyopathy is the most common pathology associated with *Trypanosoma cruzi* infection. Reports that statins have both cardioprotective and trypanocidal activity have generated interest in their potential as a therapeutic treatment. Using a highly sensitive bioluminescent mouse model, we show that a 5-day treatment with statins has no significant impact on parasite load. The free systemic concentrations fail to reach the level required for potency. Hence, clinical trials to investigate the trypanocidal activity of statins lack experimental justification.

KEYWORDS *Trypanosoma cruzi*, Chagas disease, Chagas cardiomyopathy, statin, fluvastatin, pravastatin, simvastatin, benznidazole, pharmacokinetics

Chagas disease cardiomyopathy is the major clinical manifestation of long-term infection with the protozoan parasite *Trypanosoma cruzi* and affects more than 40% of those infected. Pathology driven by persistent inflammatory responses results in a range of cardiac impairments, permanent structural changes in the myocardium, and increased mortality (1). The current consensus is that parasite persistence is necessary for the development of Chagas cardiomyopathy (2). However, the drugs currently available to treat *T. cruzi* infection have limitations in terms of efficacy, and toxic adverse effects can lead to early treatment termination (3). Crucially, benznidazole (BZ), the front-line therapeutic drug, did not reverse cardiac damage in a clinical trial (4). A drug that combines trypanocidal activity, with an ability to control host factors that mediate cardiac pathology, would be the holy grail of Chagas disease research.

Statins are a group of fungal metabolites that inhibit 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, the rate-limiting enzyme in cholesterol biosynthesis. In addition to cholesterol-lowering activity, statins have anti-inflammatory and immunomodulatory properties. They also slow blood clotting, stabilize atherosclerotic plaques, and can reduce cardiovascular disorders (5–8). These diverse systemic effects have generated interest in exploring their potential for treating infectious diseases (9). Statins are currently used by >200 million people to help lower the level of low-density lipoprotein cholesterol in the blood. Although some safety concerns have been raised, the overwhelming evidence suggests that the benefits of therapy far outweigh the risks (10). Currently, a proof-of-concept phase II clinical trial (11) is ongoing to determine if statins have a beneficial impact on inflammation and cardiac function in non-symptomatic chronically infected patients pre-treated with BZ or nifurtimox. Reports suggest that simvastatin can reduce both parasitemia and cardiac parasite burden in an acute model of Chagas disease, as well as induce anti-inflammatory responses (12). Lovastatin was also reported to be effective against *T. cruzi* epimastigotes and to potentiate the therapeutic effects of the ergosterol biosynthesis inhibitor ketoconazole (13). In contrast, although simvastatin improved cardiac remodeling in *T. cruzi*-infected dogs, it was not effective at reducing circulating parasites (14). The aim of the current work was to assess

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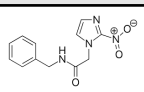
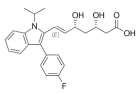
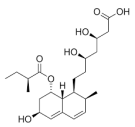
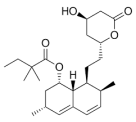
TABLE 1 *In vitro* potency of statins against *T. cruzi* epimastigotes (epis) and amastigotes (amas)[a]

	Structure	EC_{50} epis (μM)	EC_{50} amas (μM)	EC_{50} COLO-N680 (μM)	SI
BZ		4.6 ± 0.7	0.5 ± 0.2	>800	>400
F		>45	10.3 ± 2.6	430 ± 13	42
P		>47	42 ± 6	481 ± 7	12
S		27 ± 5	5.7 ± 1.2	83 ± 3	15

[a]The activity of fluvastatin (F), pravastatin (P), and simvastatin (S) was assessed against the *T. cruzi* CL Brener Luc:mNeon strain (15) by applying eight-point potency curves (16). Benznidazole (BZ) was included as a standard. Mammalian cell cytotoxicity was determined using COLO-N680 cells (human oesophageal squamous cell carcinoma). The selectivity index (SI) was the ratio of the amastigote/COLO-N680 EC_{50} values. Data were derived from two independent experiments carried out in triplicate.

different statins in a highly sensitive experimental model of Chagas disease and to investigate the extent of their *in vivo* trypanocidal activity.

First, we assessed the *in vitro* activity of fluvastatin (Lescol XL), pravastatin, and simvastatin (purchased from Novartis Pharmaceuticals, EDM Millipore Corp. and Cayman Chemical Co., respectively) against the *T. cruzi* CL Brener strain (DTU VI). Each compound was considerably less effective than BZ in blocking the growth of extracellular epimastigotes (Table 1). Similarly, activity against intracellular amastigotes, the parasite life-cycle stage that replicates in the mammalian host, was greatly inferior to that of BZ. Simvastatin was the most potent statin tested against amastigotes (EC_{50} = 5.7 μM) but was still 10 times less effective than BZ.

To assess *in vivo* efficacy, mice (aged 6–8 weeks) were infected with a strain of *T. cruzi* CL Brener engineered to express a bioluminescent fusion protein (17). At the peak of the acute stage, they were treated with five daily oral doses of fluvastatin, pravastatin, and simvastatin, at levels that simulate daily exposure at the highest human doses (18) (Fig. 1A through D). Fluvastatin, pravastatin, and simvastatin were formulated as 1.5, 5, and 9 mg/mL suspensions in 5% dimethyl sulfoxide (DMSO) and 95% hydroxypropyl methylcellulose (HPMC) (0.5% [w/v] HPMC and 0.4% [v/v] Tween 80 in Milli-Q H_2O). BZ was synthesized by Epichem Pty Ltd., Australia, and prepared at 10 mg/mL in an aqueous suspension HPMC (vehicle). None of the statin treatment schedules had any significant effect on the bioluminescence-inferred parasite burden or the parasite organ/tissue distribution post-treatment. In contrast, BZ treatment (100 mg/kg) reduced the parasite burden by 99.8%, although by 35 dpi, parasite relapse was detected in each mouse (Fig. 1C). Mice typically require 20 days of treatment with this BZ regimen to achieve sterile cure (19). Only simvastatin, the most active of the statins *in vitro* (Table 1), was tested as a treatment for chronic-stage infection. We found that there was no significant impact on the parasite burden or organ/tissue distribution after 5 days of treatment at 90 mg/kg, delivered 91–95 days post-infection (Fig. 1B through D). In contrast, treatment with BZ (100 mg/kg) reduced the parasite burden below the limit of detection. This BZ treatment schedule is generally curative when applied to chronic-stage infections (9, 10). At the experimental endpoints (100 dpi, acute stage treatment; 175 dpi, chronic stage treatment), infection foci were prominent in the gastrointestinal (GI) tract and skin, and sporadic in other organs and tissues (Fig. 1D), a pattern of distribution similar to that in non-treated mice in this infection model (17, 20).

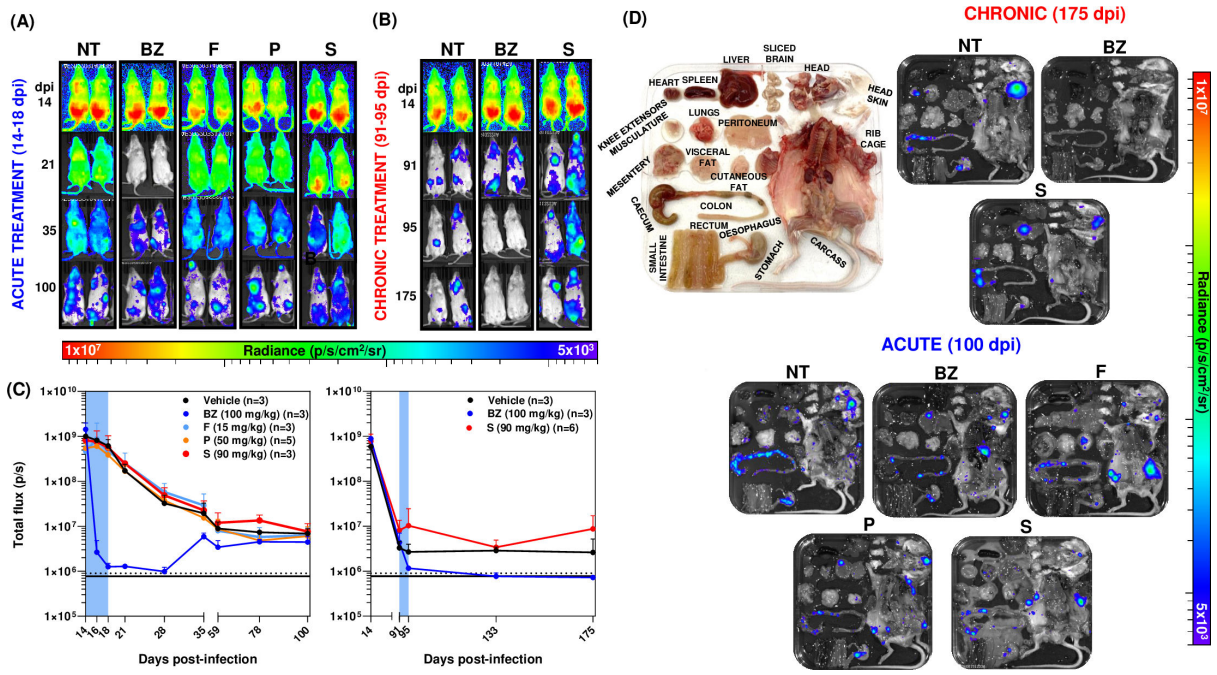


FIG 1 Statin treatment is ineffective at reducing the parasite burden during acute and chronic *T. cruzi* infections. (A and B) Representative *in vivo* ventral images of female BALB/c mice infected with 1×10^3 bloodstream trypomastigotes of the *T. cruzi* CL Brener Luc strain (19). They were treated with benznidazole and statins for 5 days, beginning 14 days post-infection (dpi) for the acute treatment and 91 dpi for the chronic treatment. Drugs were administered once daily by oral gavage. NT, non-treated (vehicle only); BZ, benznidazole-treated (100 mg/kg); F, fluvastatin-treated (15 mg/kg); P, pravastatin-treated (50 mg/kg); S, simvastatin-treated (90 mg/kg). The heat map is on a log10 scale and indicates the intensity of bioluminescence from low (blue) to high (red); the minimum and maximum radiances for the pseudocolor scale are shown. (C) Graphs showing the mean bioluminescence (pixels/second [p/s]) determined by *in vivo* imaging of treated and non-treated infected mice. Treatment groups (as above) and dosing regimens, including time of treatment (blue bar), are indicated. The black horizontal unbroken line indicates background bioluminescence established from non-infected mice (n = 3), with the dashed line indicating SD above the average. (D) Representative *ex vivo* images of tissues and organs (21) from statin-treated mice (as above) during acute and chronic infections. Mouse *ex vivo* tissue/organ arrangement is shown in the picture display. Animal experiments were performed under UK Home Office project license P9AEE04E4 and approved by the LSHTM Animal Welfare and Ethical Review Board. All procedures were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

In parallel, fluvastatin, pravastatin, and simvastatin exposure were assessed in mice during the acute stage of infection (Fig. 2). Free statin concentrations were calculated based on their respective plasma protein binding. This revealed that the unbound levels of all statins remained below the concentrations required for *in vitro* amastigote potency (Table 1) for the duration of the testing period. In contrast, the unbound BZ concentration was maintained well above the amastigote EC_{50} value throughout (Fig. 2). Furthermore, other statin pharmacokinetic parameters predictive of bioavailability and *in vivo* efficacy were inferior to those of BZ (Table 2).

Several studies have reported that statins have the potential to mitigate the development of chronic Chagas heart disease (22–24), and a clinical trial to address this is underway (11). In addition, it has been suggested that statins may have an additional benefit, conferred through their trypanocidal activity (12, 13, 25). However, the available data on *in vivo* efficacy have been contradictory. Here, using highly sensitive *in vivo* imaging employing a widely used murine model (15, 21), we demonstrate that statins have no significant impact on the parasite burden during both acute and chronic *T. cruzi* infections. Consistent with this, we show that statin bioavailability is insufficient to produce an anti-parasitic effect. Therefore, when designing clinical trials to assess the therapeutic potential of statins against Chagas disease, their use in combination with other trypanocidal drugs as adjunct therapies (as in Reference 11) represents the best evidence-based approach.

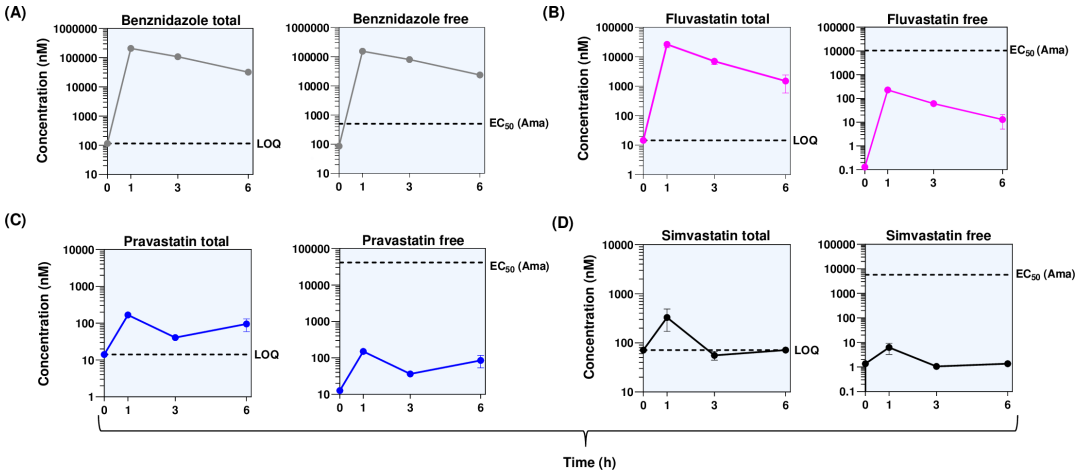


FIG 2 Systemic concentrations of statins and benznidazole during treatment of infected BALB/c mice. Drugs were administered by oral gavage at the doses described in the legend to Fig. 1. Following the last dose of acute stage treatment (day 18), blood samples were taken from the tail vein at 0 (pre-dose), 1, 3, and 6 h, placed into cryovials containing 20 µL of milli-Q water, and stored at −20°C until analysis. Samples were prepared, and analyte quantitation was performed by optimized high-performance liquid chromatography coupled with tandem mass spectrometry. A calibration curve was freshly prepared and analyzed with every set of study samples. Intra-day variability was established with triplicate quality control samples at three concentration levels. Left-hand panels show the total systemic concentrations in each case, which represent the average values from three mice. The dotted line represents the limit of quantification. (A) benznidazole, 115 nM; (B) fluvastatin, 14.6 nM; (C) pravastatin, 14.1 nM; and (D) simvastatin, 71.7 nM. The right-hand panels show mean free concentration±SD, based on their respective plasma protein binding. The dotted line identifies EC_{50} against amastigotes (Table 1).

TABLE 2 Pharmacokinetic parameters of statins in *T. cruzi* infected mice[a]

Parameters	Units	Benznidazole (100 mg/kg)	Fluvastatin (15 mg/kg)	Pravastatin (50 mg/kg)	Simvastatin (90 mg/kg)
C_{max}	µM	209	27	0.17	0.33
AUClast	µM*h	632	60	0.50	0.78
mPPB	%	26	99.1	10.2	98.1
fCmax	µM	155	0.23	0.15	0.01
fAUClast	µM*h	468	0.51	0.45	0.02

[a]Values were obtained using blood samples taken from treated female BALB/c mice (n = 3) 18 days post-infection using the doses indicated (Fig. 2). Data from benznidazole-treated mice are shown for comparison. C_{max}, maximum systemic concentration; AUC_{last}, area under the curve, 0–6 h; mPBB, mouse plasma protein binding; fC_{max}, maximum unbound systemic concentration; $fAUC_{last}$, unbound area under the curve.

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REFERENCES

1. Nascimento BR, Naback ADN, Santos BMP, Geissbühler Y, Demacq C, Quijano M, Perel PA, Molina I, Machado IE, Cousin E, Mosser JF, Carvalho P de P, Martins-Melo FR, Ribeiro ALP, RAISE investigators. 2024. Prevalence of clinical forms of Chagas disease: a systematic review and meta-analysis - data from the RAISE study. Lancet Reg Health Am 30:100681. https://doi.org/10.1016/j.lana.2024.100681
2. Bonney KM, Engman DM. 2015. Autoimmune pathogenesis of Chagas heart disease: looking back, looking ahead. Am J Pathol 185:1537–1547. https://doi.org/10.1016/j.ajpath.2014.12.023
3. Gaspar L, Moraes CB, Freitas-Junior LH, Ferrari S, Costantino L, Costi MP, Coron RP, Smith TK, Siqueira-Neto JL, McKerrow JH, Cordeiro-da-Silva A. 2015. Current and future chemotherapy for Chagas disease. Curr Med Chem 22:4293–4312. https://doi.org/10.2174/092986732266615101512 0804
4. Morillo CA, Marin-Neto JA, Avezum A, Sosa-Estani S, Rassi A Jr, Rosas F, Villena E, Quiroz R, Bonilla R, Britto C, Guhl F, Velazquez E, Bonilla L, Meeks B, Rao-Melacini P, Pogue J, Mattos A, Lazdins J, Rassi A, Connolly SJ, Yusuf S. 2015. Randomized trial of benznidazole for chronic chagas' cardiomyopathy. N Engl J Med 373:1295–1306. https://doi.org/10.1056/ NEJMoa1507574
5. Hill JS, Qiu G. 2008. Understanding how statins work: the path to better treatments for heart disease and more. Future Cardiol 4:5–8. https://doi. org/10.2217/14796678.4.1.5
6. Weitz-Schmidt G. 2002. Statins as anti-inflammatory agents. Trends Pharmacol Sci 23:482–486. https://doi.org/10.1016/s0165-6147(02)0207 7-1
7. Fernández-Ruiz I. 2022. Statins promote efferocytosis in atherosclerotic plaques. Nat Rev Cardiol 19:286–286. https://doi.org/10.1038/s41569-02 2-00699-5
8. Undas A, Brummel-Ziedins KE, Potaczek DP, Stobierska-Dzierzek B, Bryniarski L, Szczeklik A, Mann KG. 2006. Atorvastatin and quinapril inhibit blood coagulation in patients with coronary artery disease following 28 days of therapy. J Thromb Haemost 4:2397–2404. https://d oi.org/10.1111/j.1538-7836.2006.02165.x
9. Hennessy E, Adams C, Reen FJ, O'Gara F. 2016. Is there potential for repurposing statins as novel antimicrobials? Antimicrob Agents Chemother 60:5111–5121. https://doi.org/10.1128/AAC.00192-16
10. Adhyaru BB, Jacobson TA. 2018. Safety and efficacy of statin therapy. Nat Rev Cardiol 15:757–769. https://doi.org/10.1038/s41569-018-0098-5
11. Campos-Estrada C, Urarte E, Denegri M, Villalón L, González-Herrera F, Kemmerling U, Maya JD. 2023. Effect of statins on inflammation and cardiac function in patients with chronic Chagas disease: a protocol for pathophysiological studies in a multicenter, placebo-controlled, proof-of-concept phase II trial. PLoS One 18:e0280335. https://doi.org/10.1371 /journal.pone.0280335
12. Silva RR, Shrestha-Bajracharya D, Almeida-Leite CM, Leite R, Bahia MT, Talvani A. 2012. Short-term therapy with simvastatin reduces inflammatory mediators and heart inflammation during the acute phase of experimental Chagas disease. Mem Inst Oswaldo Cruz 107:513–521. h ttps://doi.org/10.1590/s0074-02762012000400012
13. Urbina JA, Lazardi K, Marchan E, Visbal G, Aguirre T, Piras MM, Piras R, Maldonado RA, Payares G, de Souza W. 1993. Mevinolin (lovastatin) potentiates the antiproliferative effects of ketoconazole and terbinafine against *Trypanosoma* (*Schizotrypanum*) cruzi: *in vitro* and *in vivo* studies. Antimicrob Agents Chemother 37:580–591. https://doi.org/10.1128/AAC .37.3.580
14. Melo L, Caldas IS, Azevedo MA, Gonçalves KR, da Silva do Nascimento AF, Figueiredo VP, de Figueiredo Diniz L, de Lima WG, Torres RM, Bahia

MT, Talvani A. 2011. Low doses of simvastatin therapy ameliorate cardiac inflammatory remodeling in *Trypanosoma cruzi*-infected dogs. Am J Trop Med Hyg 84:325–331. https://doi.org/10.4269/ajtmh.2011.10-0451
15. Costa FC, Francisco AF, Jayawardhana S, Calderano SG, Lewis MD, Olmo F, Beneke T, Gluenz E, Sunter J, Dean S, Kelly JM, Taylor MC. 2018. Expanding the toolbox for *Trypanosoma cruzi*: a parasite line incorporating a bioluminescence-fluorescence dual reporter and streamlined CRISPR/Cas9 functionality for rapid *in vivo* localisation and phenotyping. PLoS Negl Trop Dis 12:e0006388. https://doi.org/10.1371/journal.pntd.0006388
16. Scarim CB, Olmo F, Ferreira EI, Chin CM, Kelly JM, Fortes Francisco A. 2021. Image-based *in vitro* screening reveals the trypanostatic activity of hydroxymethylnitrofurazone against *Trypanosoma cruzi*. Int J Mol Sci 22:6930. https://doi.org/10.3390/ijms22136930
17. Lewis MD, Fortes Francisco A, Taylor MC, Burrell-Saward H, McLatchie AP, Miles MA, Kelly JM. 2014. Bioluminescence imaging of chronic *Trypanosoma cruzi* infections reveals tissue-specific parasite dynamics and heart disease in the absence of locally persistent infection. Cell Microbiol 16:1285–1300. https://doi.org/10.1111/cmi.12297
18. Dutta NK, Bruiners N, Zimmerman MD, Tan S, Dartois V, Gennaro ML, Karakousis PC. 2020. Adjunctive host-directed therapy with statins improves tuberculosis-related outcomes in mice. J Infect Dis 221:1079–1087. https://doi.org/10.1093/infdis/jiz517
19. Francisco AF, Jayawardhana S, Lewis MD, White KL, Shackleford DM, Chen G, Saunders J, Osuna-Cabello M, Read KD, Charman SA, Chatelain E, Kelly JM. 2016. Nitroheterocyclic drugs cure experimental *Trypanosoma cruzi* infections more effectively in the chronic stage than in the acute stage. Sci Rep 6:35351. https://doi.org/10.1038/srep35351
20. Ward AI, Lewis MD, Khan AA, McCann CJ, Francisco AF, Jayawardhana S, Taylor MC, Kelly JM. 2020. *In vivo* analysis of *Trypanosoma cruzi* persistence foci at single cell resolution. MBio 11:e01242-20. https://doi.org/10.1128/mBio.01242-20
21. Lewis MD, Francisco AF, Taylor MC, Kelly JM. 2015. A new experimental model for assessing drug efficacy against *Trypanosoma cruzi* infection based on highly sensitive *in vivo* imaging. J Biomol Screen 20:36–43. https://doi.org/10.1177/1087057114552623
22. Guzmán-Rivera D, Liempi A, González-Herrera F, Fuentes-Retamal S, Carrillo I, Abarca P, Castillo C, Kemmerling U, Pesce B, Maya JD. 2020. Simvastatin improves cardiac function through Notch 1 activation in BALB/c mice with chronic Chagas cardiomyopathy. Antimicrob Agents Chemother 64:e02141-19. https://doi.org/10.1128/AAC.02141-19
23. González-Herrera F, Cramer A, Pimentel P, Castillo C, Liempi A, Kemmerling U, Machado FS, Maya JD. 2017. Simvastatin attenuates endothelial activation through 15-epi-lipoxin A4 production in murine chronic Chagas cardiomyopathy. Antimicrob Agents Chemother 61:e02137-16. https://doi.org/10.1128/AAC.02137-16
24. González-Herrera F, Clayton NS, Guzmán-Rivera D, Carrillo I, Castillo C, Catalán M, Anfossi R, Quintero-Pertuz H, Quilaqueo ME, Olea-Azar C, Rivera-Meza M, Kemmerling U, Ridley AJ, Vivar R, Maya JD. 2022. Statins change the cytokine profile in *Trypanosoma cruzi*-infected U937 macrophages and murine cardiac tissue through Rho-associated kinases inhibition. Front Immunol 13:1035589. https://doi.org/10.3389/fimmu.2022.1035589
25. Araujo-Lima CF, de Cassia Castro Carvalho R, Rosario SL, Leite DI, Aguiar ACC, de Souza Santos LV, de Araujo JS, Salomão K, Kaiser CR, Krettli AU, Bastos MM, Aiub CAF, de Nazaré Correia Soeiro M, Boechat N, Felzenszwalb I. 2023. Antiplasmodial, trypanocidal, and genotoxicity *in vitro* assessment of new hybrid α,α-difluorophenylacetamide-statin derivatives. Pharmaceuticals (Basel) 16:782. https://doi.org/10.3390/ph16060782