

Anti-*Trypanosoma cruzi* activity of the anti-arrhythmic compound amiodarone: in vitro, in vivo and 3D-QSAR studies

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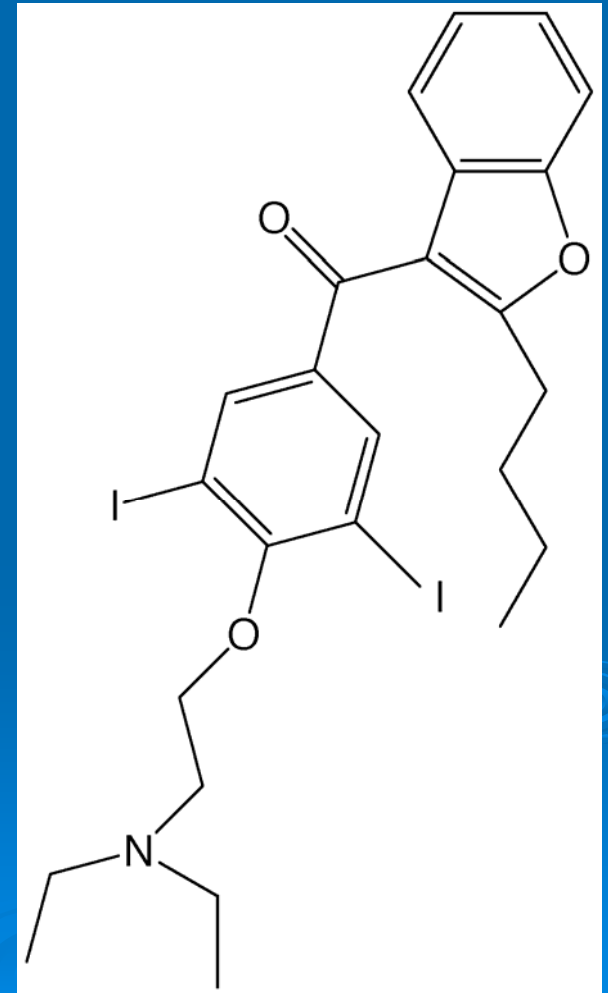
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Switzerland

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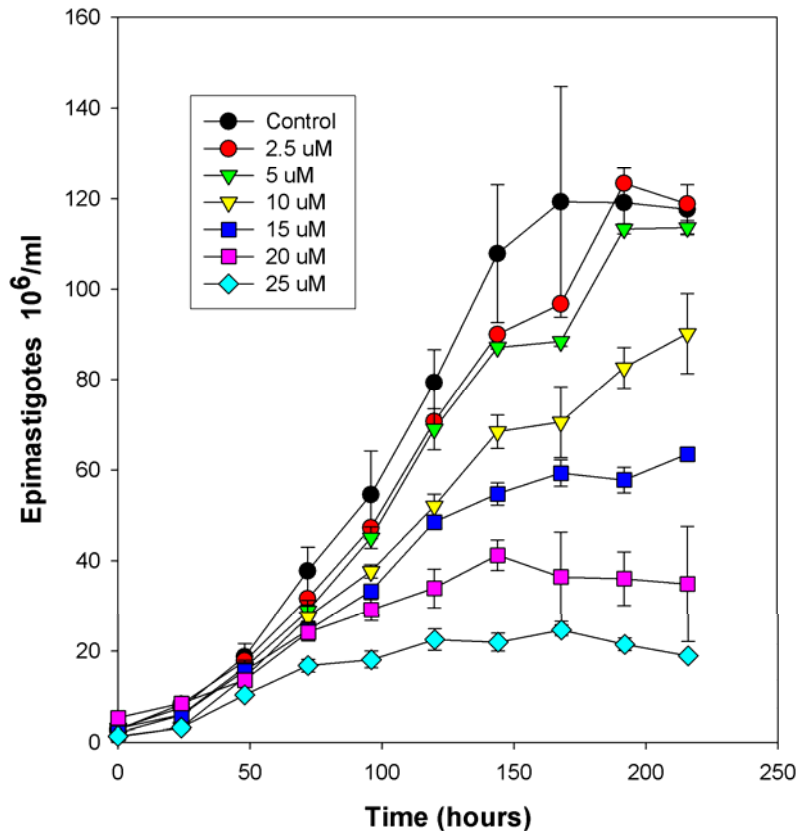
Amiodarone is the most frequently used anti-arrhythmic agent in Chagas disease patients with cardiac compromise

- The mechanism of action involves a direct blockade of Ca^{2+} plasmalemmal channels, a shift of the inactivation potential to more negative values and an increase of the refractory period. Also, non-competitive inhibition of both α and β adrenergic receptors seems to be involved
- The compound has unusual pharmacokinetic properties: tissue levels are 100 to 1000-fold higher than those in sera and the terminal elimination half life is ≥ 20 days
- Recent work has shown that this compound also has broad-spectrum antifungal activity, which is mediated by interference with the cells' Ca^{2+} homeostasis

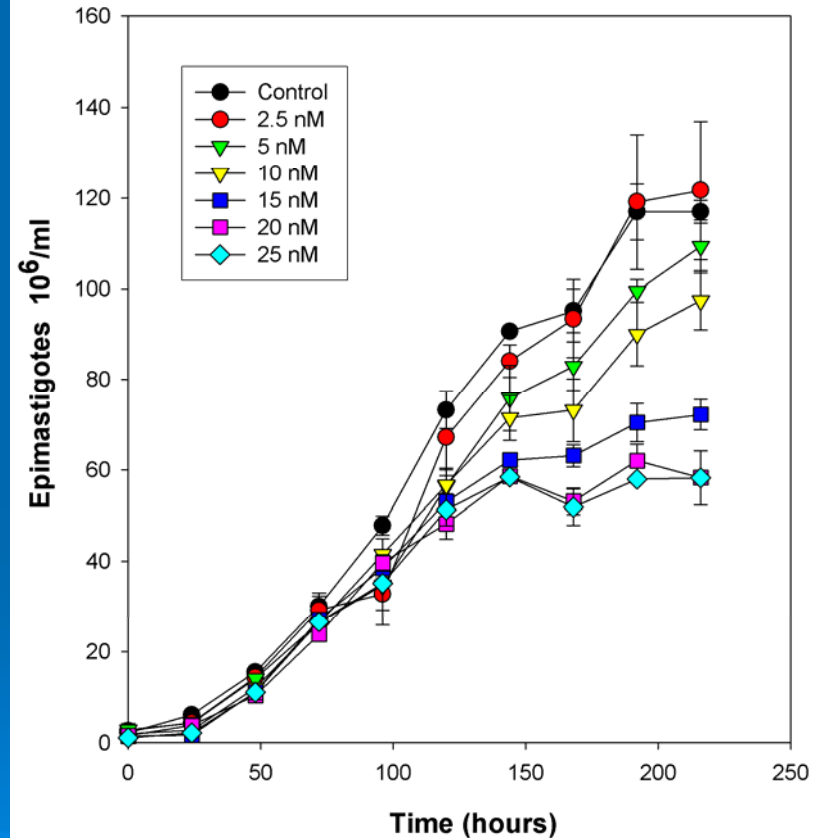


Antiproliferative activities of amiodarone and posaconazole against *T. cruzi* epimastigotes

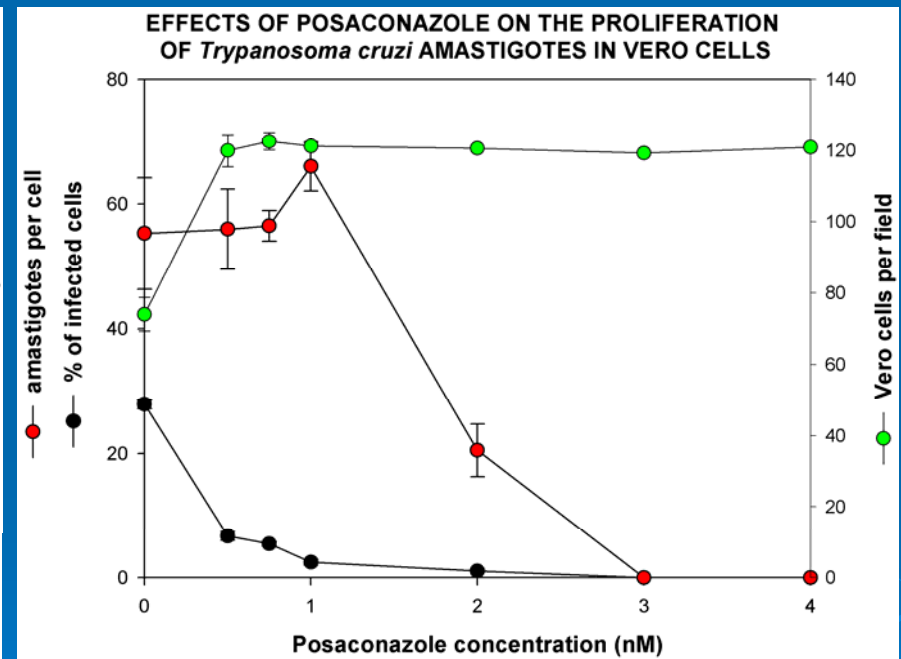
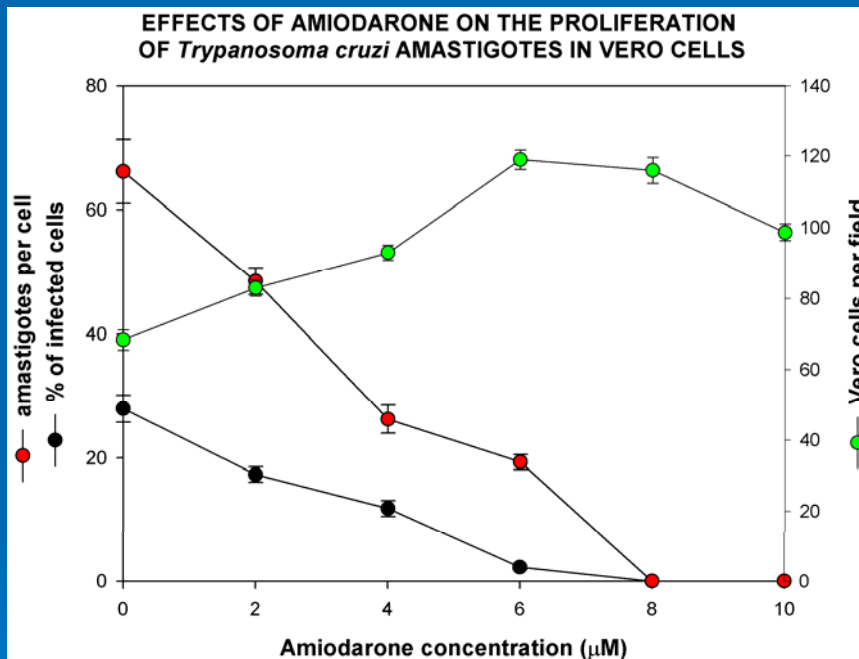
Effects of Amiodarone on *T. cruzi* epimastigotes



Effects of Posaconazole on *T. cruzi* epimastigotes

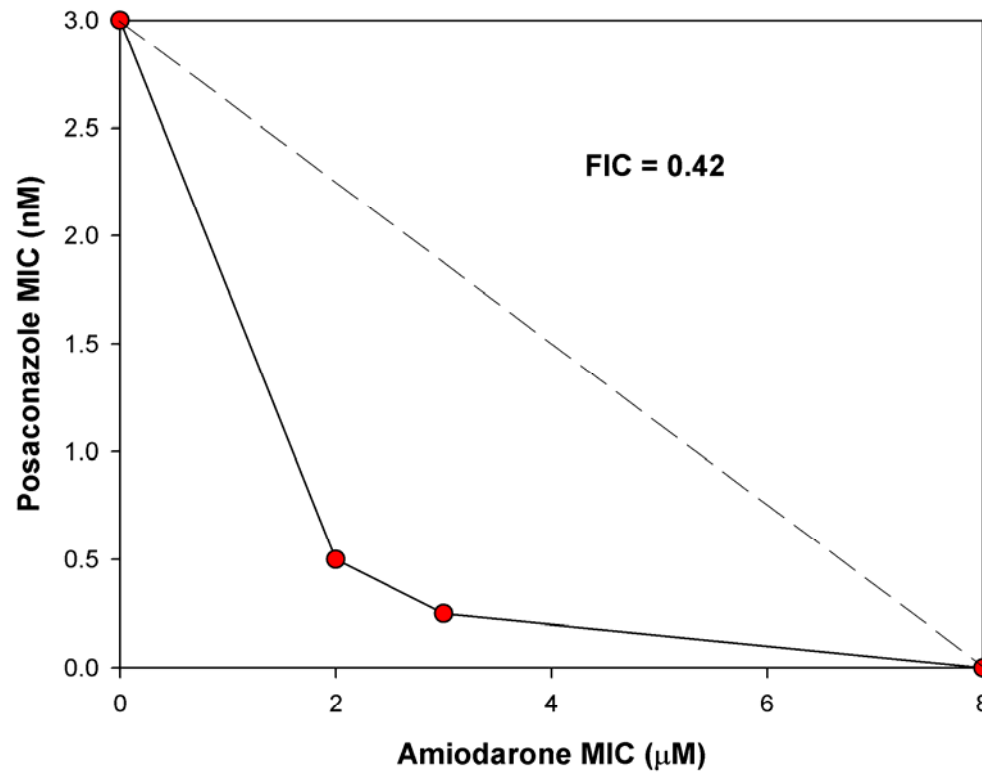


Antiproliferative activities of amiodarone and posaconazole against *T. cruzi* amastigotes



Antiproliferative synergism of amiodarone and posaconazole against *T. cruzi* amastigotes

ISOBOLOGRAM OF THE COMBINED EFFECTS OF POSACONAZOLE AND AMIODARONE ON *Trypanosoma cruzi* INTRACELLULAR AMASTIGOTES



—●— Amiodarone vs Posaconazole

Effects of amiodarone and posaconazole on free Ca^{2+} levels in *T. cruzi* epimastigotes

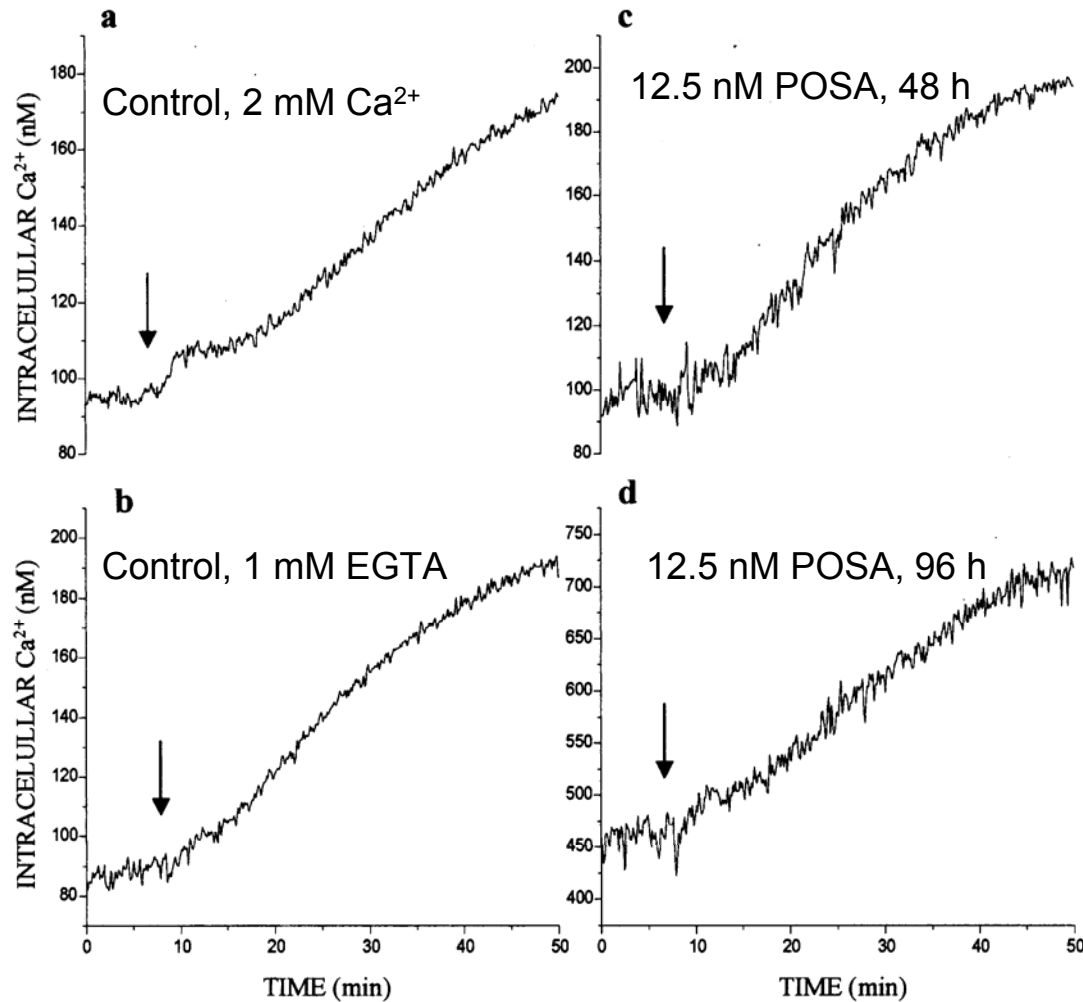


Table 1. Effects of amiodarone and posaconazole on the free cytoplasmatic Ca^{2+} concentration of *Trypanosoma cruzi* epimastigotes^a

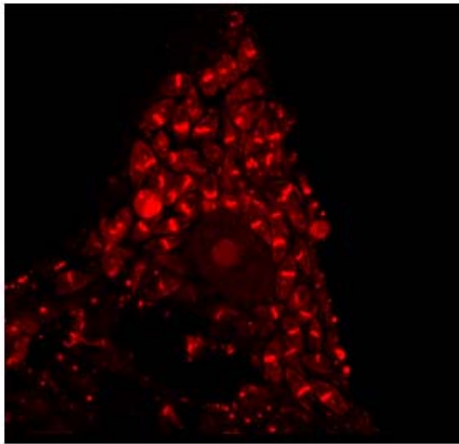
Measurement conditions	Pretreatment		
	Control (untreated)	Posaconazole 12.5 nM, 48 h	Posaconazole 12.5 nM, 96 h
2 mM $[\text{Ca}^{2+}]_{\text{ext}}$	95.0 \pm 35.02 n=15	86.3 \pm 26.7 n=8	461.4 \pm 63,8 n=9
2 mM $[\text{Ca}^{2+}]_{\text{ext}}$ + 12.5 μM amiodarone	173.9 \pm 38.5 n=6	192.9 \pm 38.9 n=4	667.8 \pm 49,8 n=4
0 mM $[\text{Ca}^{2+}]_{\text{ext}}$	99.0 \pm 10.5 n=4	97.1 \pm 15.2 n= 4	480 \pm 39,8,9 n= 4
0 mM $[\text{Ca}^{2+}]_{\text{ext}}$ + 12.5 μM amiodarone	192.5 \pm 26.2 n=4	131.2 \pm 10.8 n=4	610.9 \pm 77,4 n=4

^aFree cytoplasmatic calcium concentration were determined using fluorimetric methods using Fura-2, as described in reference 11. Concentrations are expressed in nM.

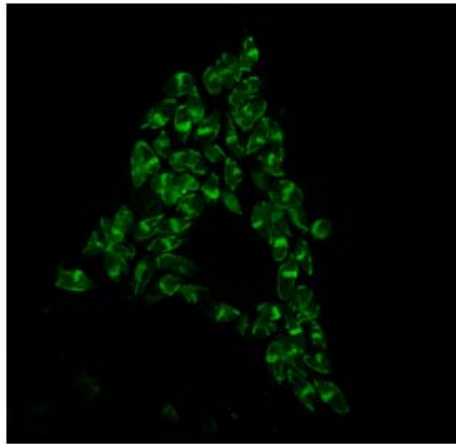
Effects of amiodarone and posaconazole on free Ca^{2+} levels in *T. cruzi* amastigotes

C

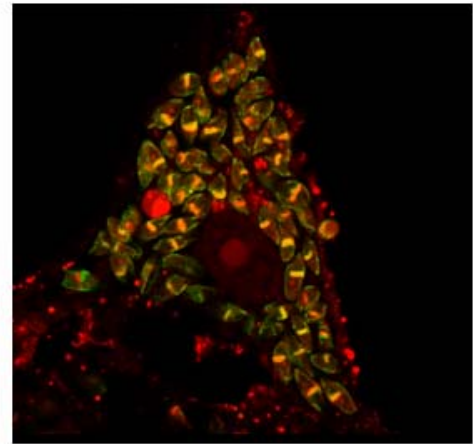
Rhod-2



Rhodamine 123



Merge



A

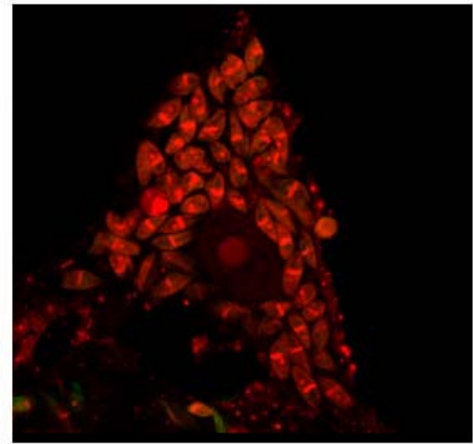
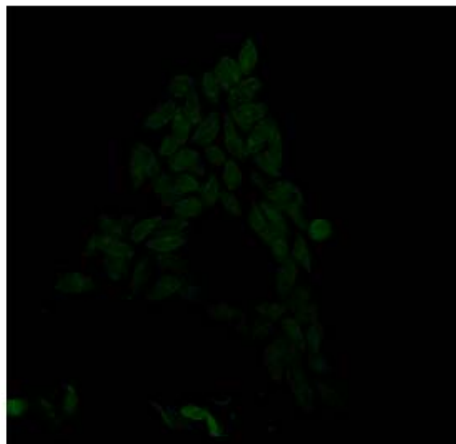
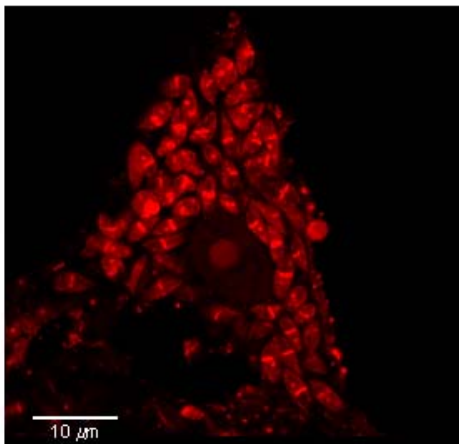
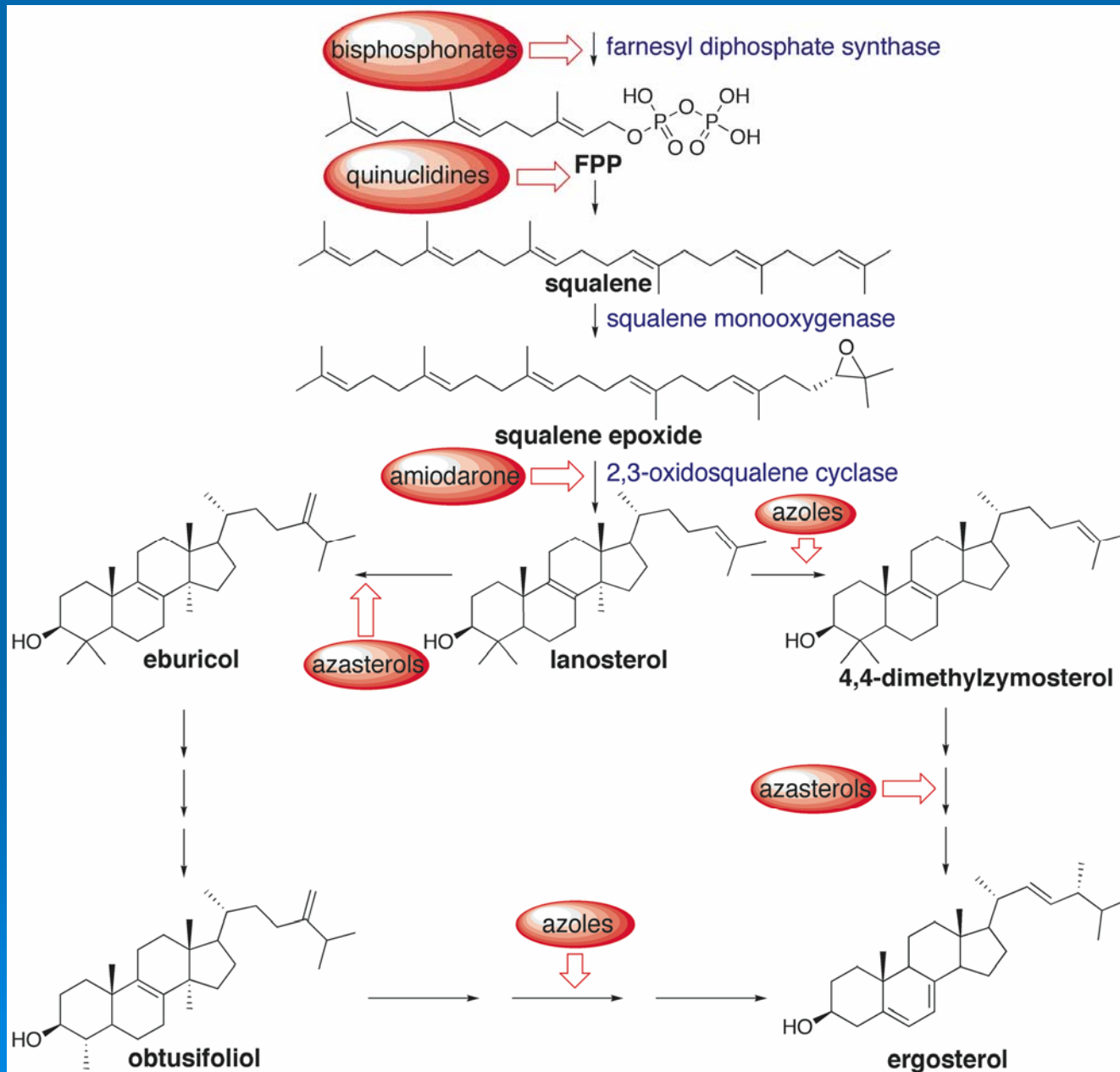


Table 2. Free sterols present in *Trypanosoma cruzi* epimastigotes (EP stock) grown in the absence or presence of posaconazole, amiodarone or their combination^a

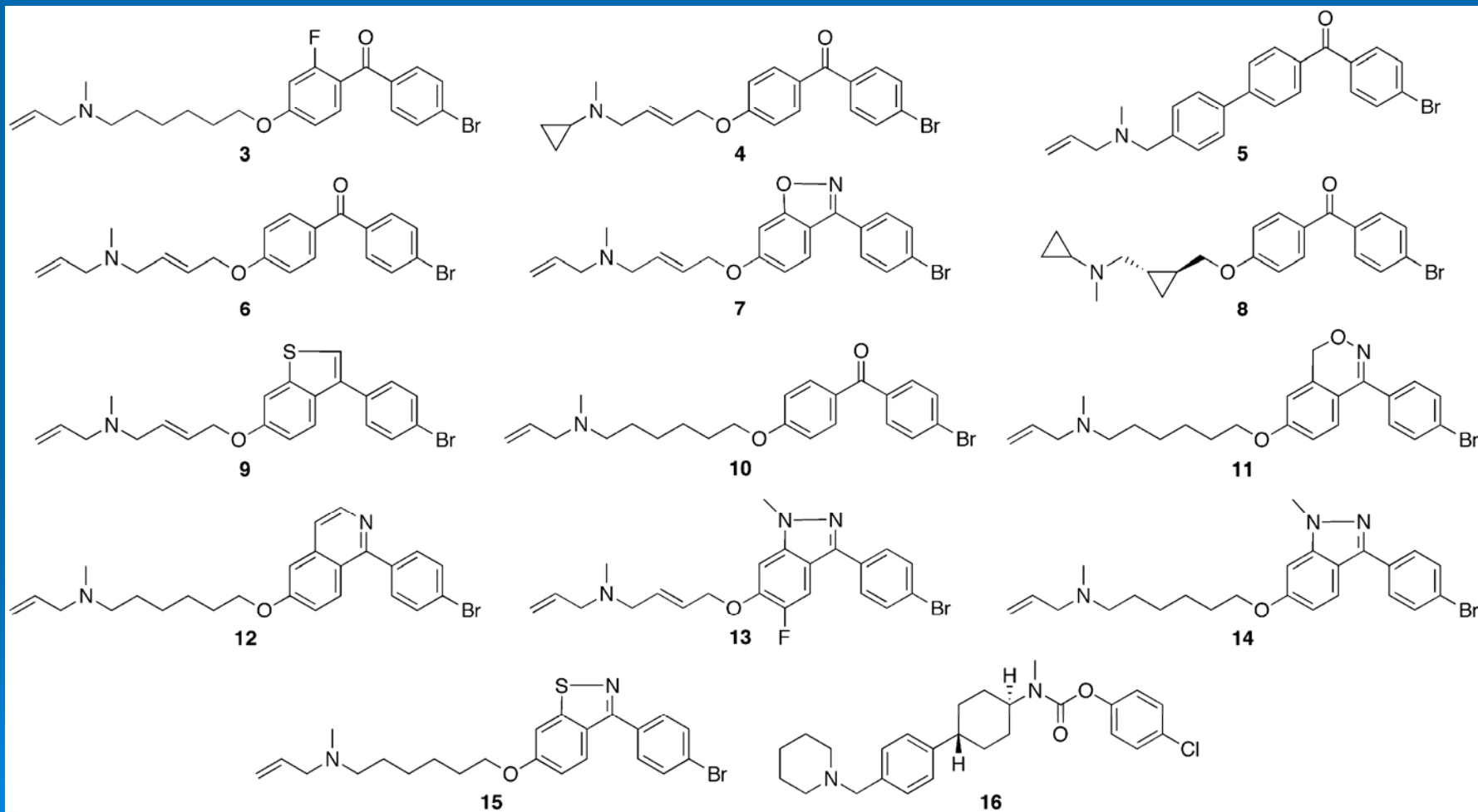
Sterol^c	Control	Posaconazole 12.5 nM	Amiodarone 12.5 μM	Posaconazole 12.5 nM+ Amiodarone 12.5 μM
Exogenous Cholesterol	31.2	46.1	63.4	78.8
<i>Endogenous, 14-desmethyl:</i>				
Ergosterol	15.3	5.1	5.1	n.d. ^d
24-ethyl-5,7,22-cholesta-trien-3b-ol	15.4	12.2	6.7	n.d.
Ergosta-8,24(24')-dien-3b-ol	6.5	13.7	10.2	n.d.
Ergosta-5,7-dien-3b-ol	9.5	3.6	4.1	n.d.
Ergosta-5,7,24(24')-trien-3b-ol	8.5	n.d.	n.d.	n.d.
Ergosta-7,24(24')-dien-3b-ol	7.6	3.2	3.0	n.d.
24-ethyl-5,7-cholesta-dien-3b-ol	6.0	7.5	7.5	n.d.
<i>Endogenous, 14-methyl:</i>				
24-methylenedihydrolanosterol	n.d.	3.2	n.d.	16.9
Lanosterol	n.d.	5.5	n.d.	4.3

^cFree sterols were isolated and purified from whole cells grown in the absence or presence of the indicated drug concentrations for 96 h; they were analyzed by high resolution gas-liquid chromatography coupled with mass spectrometry, as described in Materials and Methods.

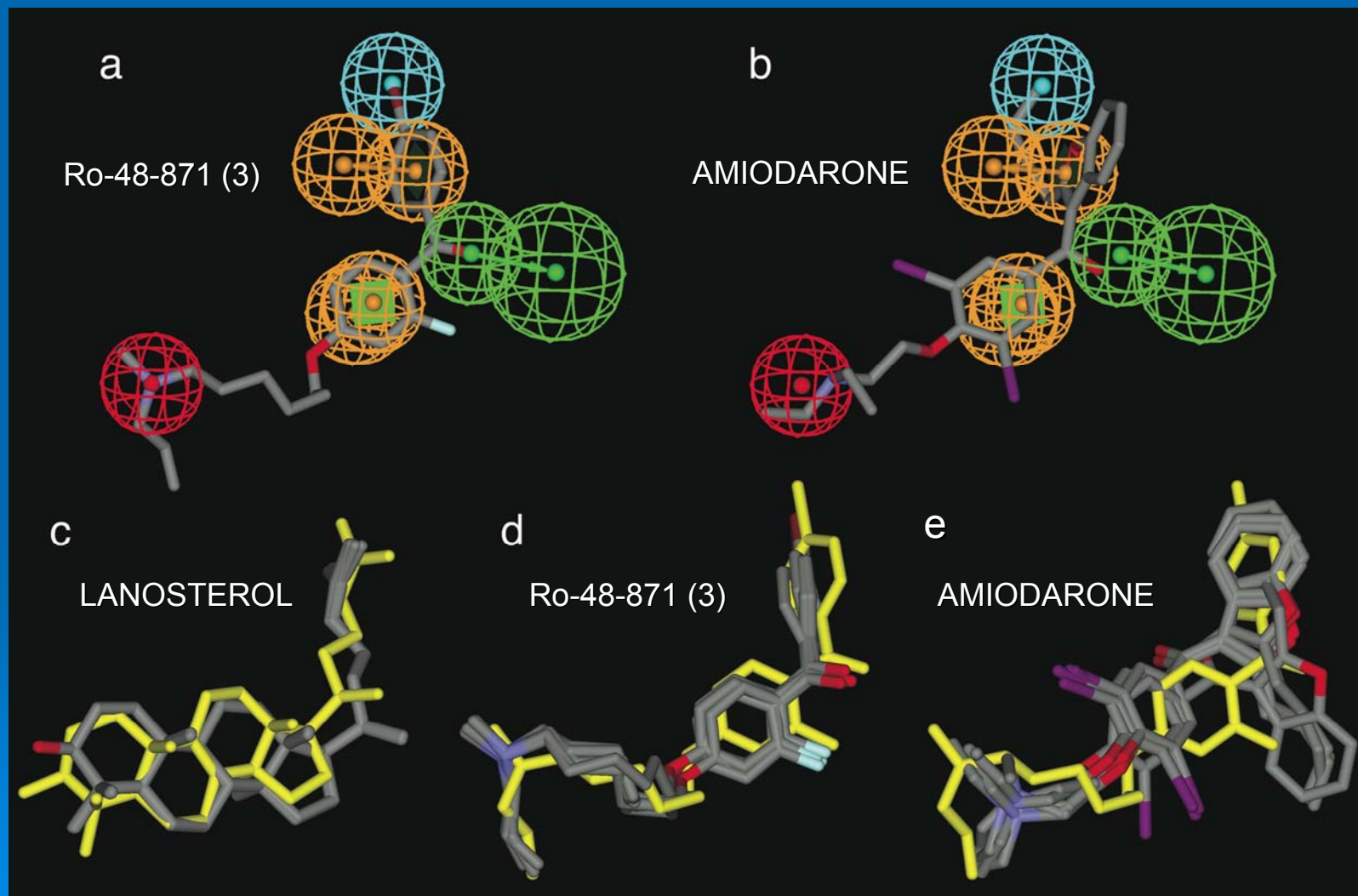
^dn.d., not detected.



3D-QSAR studies of oxido-squalene cyclase (OSC) inhibitors: training set



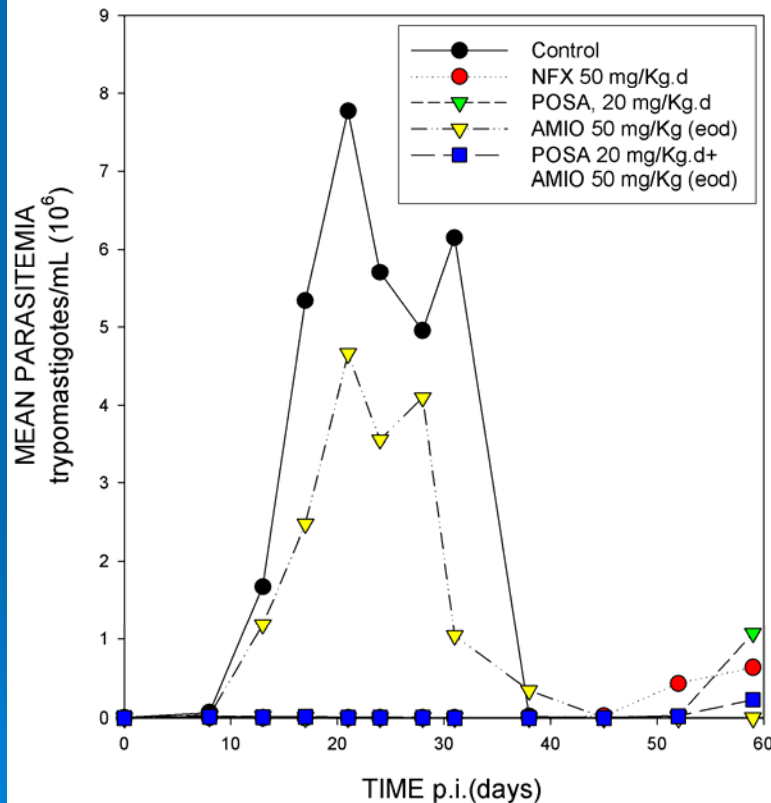
Catalyst pharmacophore and molecular docking for oxido-squalene cyclase (OSC) inhibition



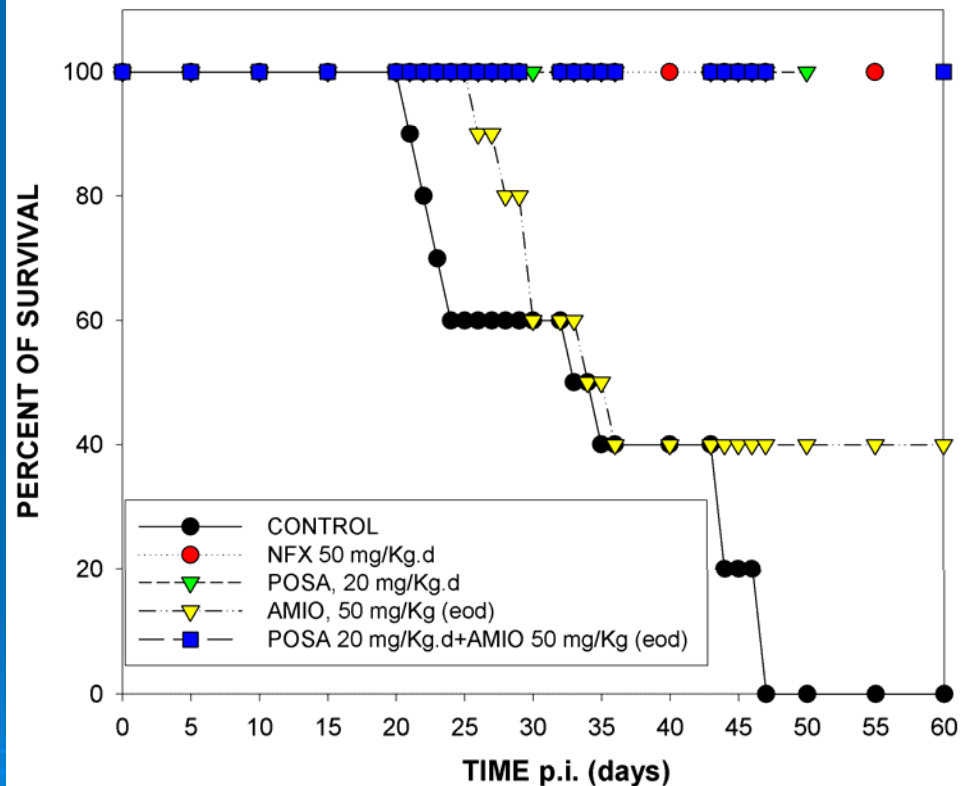
Activities of amiodarone and posaconazole in a murine model of acute Chagas disease

10^5 trypomastigotes/mouse

EFFECTS OF POSACONAZOLE, AMIODARONE, NIFURTIMOX AND COMBINATIONS
IN A MURINE MODEL OF ACUTE CHAGAS DISEASE,
 10^5 TRYPOMASTIGOTES PER MOUSE, Y STOCK



EFFECTS OF POSACONAZOLE, AMIODARONE, NIFURTIMOX AND COMBINATIONS
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 10^5 TRYPOMASTIGOTES PER MOUSE, Y STOCK



Activities of amiodarone and posaconazole in a murine model of acute Chagas disease

10³ trypomastigotes/mouse

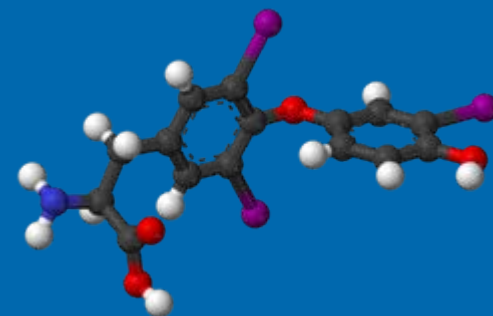
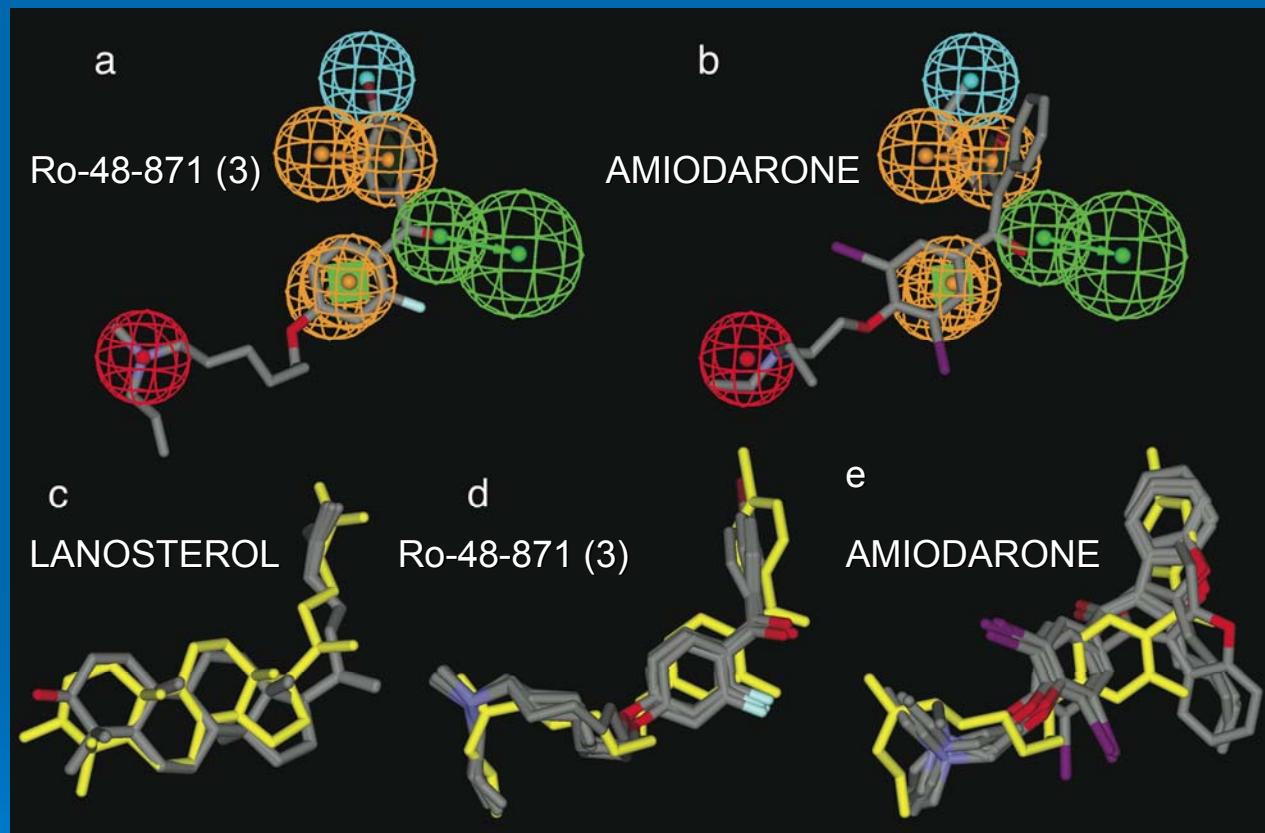
Table 3. Effects of amiodarone and posaconazole in a murine model of acute Chagas disease. 10³ trypomastigotes mouse⁻¹, Y strain, 86 days p.i.^a.

Treatment conditions	Survival	Negative parasitological tests ^b
No treatment (control)	8/10	3/8
Amiodarone, 50 mg kg ⁻¹ , e.o.d., 15 doses	6/10	0/6
Posaconazole, 20 mg kg ⁻¹ d ⁻¹ , 30 doses	10/10	6/10
Amiodarone, 50 mg kg ⁻¹ , e.o.d., 15 doses + Posaconazole, 20 mg kg ⁻¹ d ⁻¹ , 30 doses	10/10	8/10
Nifurtimox, 50 mg kg ⁻¹ d ⁻¹ , 30 doses	8/10	4/8

^aFemale NMRI-IVIC mice (20-25g) were infected with 10³ bloodstream trypomastigotes and drug treatment started 24 hours later, at the doses and frequencies indicated.

^bHemoculture, xenodiagnosis and TcZ DNA PCR, see Materials and Methods.

Amiodarone interference with thyroid function: room for improvement



TRIIODO-THYRONINE (T3)

Conclusions

- ▶ Amiodarone has an a previously unknown **intrinsic anti-*T. cruzi* activity** and acts **synergistically** with posaconazole, in vitro and in vivo
- ▶ These effects are mediated by two mutually reinforcing molecular mechanisms: **interference with the parasite's Ca^{2+} homeostasis** and **de novo ergosterol biosynthesis, at the level oxido-squalene cyclase (OSC; lanosterol synthase)**
- ▶ 3D-QSAR studies indicate that the **amiodarone structure contains the key features common to OSC inhibitors**, suggesting that it could represent a novel class of OSCI
- ▶ The results suggest that symptomatic treatment of chronic Chagas disease patients may have the added benefit of **reducing the parasite's burden and increasing the efficacy of specific antiparasitic treatments**

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