



SQ109 inhibits proliferation of *Leishmania donovani* by disruption of intracellular Ca^{2+} homeostasis, collapsing the mitochondrial electrochemical potential ($\Delta\Psi_m$) and affecting acidocalcisomes

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Abstract

Leishmania donovani is the causative agent of visceral leishmaniasis. Annually, 500 million new cases of infection are reported mainly in poor communities, decreasing the interest of the pharmaceutical industries. Therefore, the repositioning of new drugs is an ideal strategy to fight against these parasites. SQ109, a compound in phase IIb/III of clinical trials to treat resistant *Mycobacterium tuberculosis*, has a potent effect against *Trypanosoma cruzi*, responsible for Chagas' disease, and on *Leishmania mexicana*, the causative agent of cutaneous and muco-cutaneous leishmaniasis. In the latter, the toxic dose against intramacrophagic amastigotes is very low ($\text{IC}_{50} \sim 11$ nM). The proposed mechanism of action on *L. mexicana* involves the disruption of the parasite intracellular Ca^{2+} homeostasis through the collapse of the mitochondrial electrochemical potential ($\Delta\Psi_m$). In the present work, we show a potent effect of SQ109 on *L. donovani*, the parasite responsible for visceral leishmaniasis, the more severe and uniquely lethal form of these infections, obtaining a toxic effect on amastigotes inside macrophages even lower to that obtained in *L. mexicana* (IC_{50} of 7.17 ± 0.09 nM) and with a selectivity index > 800 , even higher than in *L. mexicana*. We also demonstrated for first time that SQ109, besides collapsing $\Delta\Psi_m$ of the parasite, induced a very rapid damage to the parasite acidocalcisomes, essential organelles involved in the bioenergetics and many other important functions, including Ca^{2+} homeostasis. Both effects of the drug on these organelles generated a dramatic increase in the intracellular Ca^{2+} concentration, causing parasite death.

Keywords *Leishmania donovani* · Visceral leishmaniasis · SQ109 · Anti-tuberculosis drugs · Calcium · Mitochondria · Acidocalcisomes

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Introduction

Visceral leishmaniasis (VL), also known as kala-azar (“black fever”), is a fatal protozoan disease caused by two species of trypanosomatids from the genus *Leishmania*, *Leishmania donovani* and *Leishmania infantum* (Ready 2014). With a mortality rate of 90% if not treated, VL is responsible for more than 50,000 deaths and approximately 200,000 to 500,000 new cases of infection each year, making it the second largest parasitic killer in the world after malaria (Al-Salem et al. 2016). Most VL cases occur in underdeveloped and low-income rural and suburban areas of Brazil, Ethiopia, India, Kenya, Somalia, South Sudan, and Sudan (Alvar et al. 2012). Due to the lack of sufficient research funding and adequate attention by the general public, it has been classified as

a neglected tropical disease (NTP) by the World Health Organization (WHO 2003).

Since the 1940s until the start of this century, drug therapies based on pentavalent antimonials, such as sodium stibogluconate (Pentostam®, GSK) and meglumine antimoniate (Glucantime®, Aventis), have been used to treat VL (Jyotsna et al. 2007). Pentavalent antimonials are still employed in some countries, but they are mainly considered obsolete due to their high toxicity and low effectiveness against resistant leishmaniasis (Frézard et al. 2009). Currently, the two first lines of treatment for VL are amphotericin B and miltefosine (Menez et al. 2006), drugs previously used for fungal infections (Dutcher 1968; Sawaya et al. 1995) and breast cancer (Rakotomanga et al. 2007), respectively. While amphotericin B interacts preferentially with ergosterol present in the parasite membranes forming unspecific channels, the mechanisms of action of miltefosine appear to be through the blockade of phosphatidylcholine biosynthesis (Urbina 2006) and by the disruption of the intracellular Ca^{2+} homeostasis (Pinto-Martinez et al. 2018a, 2018b; Rodriguez-Duran et al. 2019), opening a sphingosine-activated Ca^{2+} channel at the plasma membrane and also involving mitochondria and acidocalcisome damage, thus inducing a large increase in the intracellular Ca^{2+} concentration, deleterious for these parasites. Trypanosomatids are capable of maintaining an intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) of approximately 50 nM, while facing an extracellular Ca^{2+} concentration in the millimolar range (Benaïm and Romero 1990), i.e., 4 orders of magnitude larger. Three organelles, the endoplasmatic reticulum, the large mitochondrion, and acidocalcisomes, are involved in the regulation of the $[\text{Ca}^{2+}]_i$ in these microorganisms (Benaïm and Garcia 2011). These last two, in addition, are involved in parasite bioenergetics. In *Leishmania* parasites, Ca^{2+} participates in essential functions, like flagellar motion, mitochondrial oxidative metabolism, and macrophage invasion (Misra et al. 1991; Benaïm and Garcia 2011). Therefore, disrupting the $[\text{Ca}^{2+}]_i$ is a promising strategy against leishmaniasis (Benaïm and Garcia 2011). Accordingly, amiodarone, a common anti-arrhythmic drug, possesses potent trypanocidal (Benaïm et al. 2006) and leishmanicidal (Serrano-Martín et al. 2009a, 2009b) action, its main mechanism of action being the disruption of parasite Ca^{2+} homeostasis by collapsing the mitochondrial electrochemical membrane potential ($\Delta\Psi_m$) and impairing acidocalcisome function. Similarly, dronedarone, an amiodarone derivative with fewer side effects, also exerts its anti-parasitic effect in *Trypanosoma cruzi* (Benaïm et al. 2012; Benaïm and Paniz-Mondolfi 2012) and in *Leishmania mexicana* (Benaïm et al. 2014) by the same mechanism of action. Figure 1 depicts the chemical structures of leishmanicidal compounds whose mode of action involves disruption of the cellular Ca^{2+} homeostasis. Furthermore, some benzofurane derivatives recently described also have

trypanocidal (Pinto-Martinez et al. 2018a, 2018b) and leishmanicidal (Martinez-Sotillo et al. 2019) action essentially by the same mechanism.

SQ109 is a lipophilic base with an ethylene diamine structure- analog of ethambutol containing N-geranyl and N-adamantyl groups (Sacksteder et al. 2012; Borisov et al. 2018). Interestingly, SQ109 completed phases IIB/III of clinical trials for the treatment of drug-resistant tuberculosis and at the same time has been shown to affect not only bacterial, but also fungal and protozoan pathogens. Some examples include *Candida albicans* (Sacksteder et al. 2012), *Helicobacter pylori* (Makobongo et al. 2013), and *Plasmodium falciparum* (Li et al. 2014). More recently, SQ109 has been proven to be effective also on *Trypanosoma cruzi* (Veiga-Santos et al. 2015), the causative agent of Chagas disease. Concerning leishmaniasis, a recent report demonstrates a potent effect on *Leishmania mexicana* (García-García et al. 2016) with very low IC_{50} ($\text{IC}_{50} \sim 11$ nM) on amastigotes inside macrophages, the clinically relevant phase of this parasite. The mechanism of action on *L. mexicana* was shown to be the dissipation of the mitochondrial electrochemical membrane potential ($\Delta\Psi_m$) (García-García et al. 2016). In consequence, SQ109 produces a large increase in the $[\text{Ca}^{2+}]_i$ of these parasites.

In this work, we evaluated the effect of SQ109 against *L. donovani*, the causative agent of visceral leishmaniasis, the lethal form of this infectious disease, finding an anti-proliferative effect of the drug against both life cycle stages of the parasite, promastigotes and amastigotes. *L. donovani* is far more difficult to fight because these parasites, different to *L. mexicana*, are more resistant. For example, they are capable of surviving inside macrophages at much higher temperatures, since in visceral leishmaniasis, the fever reaches typically around 40 °C, while in cutaneous leishmaniasis, at the lesion, temperature oscillates between 26 and 32 °C (McCall et al. 2013). Viscerotropic parasites must therefore withstand higher temperatures than cutaneous ones, and in fact, promastigotes from cutaneous species are significantly more sensitive to heat shock than promastigotes of visceral species (Ma;Coy et al. 2013)

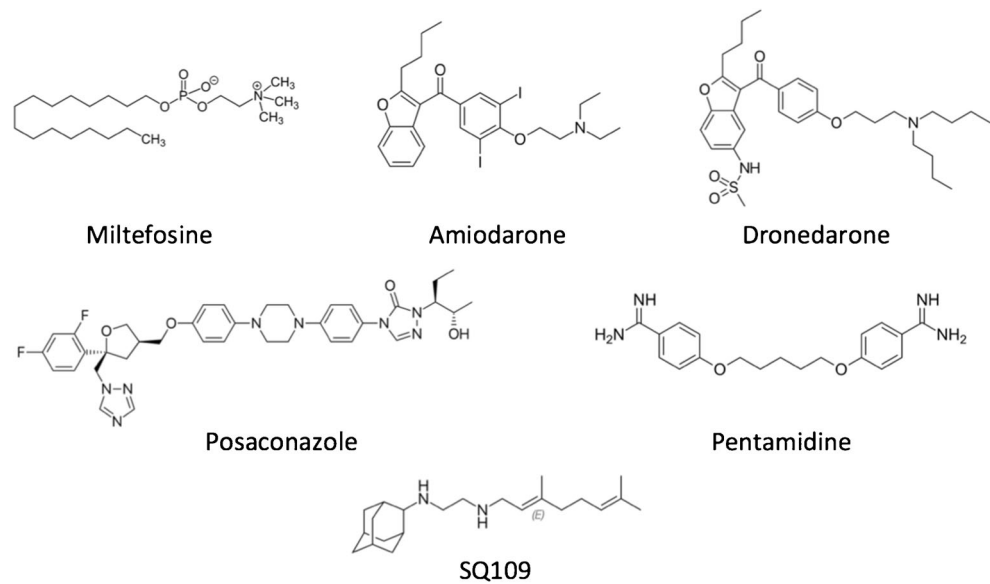
Interestingly, SQ109 was more potent against *L. donovani* than against *L. mexicana* amastigotes and with a superior selectivity index ($\text{SI} > 800$). We also demonstrated that SQ109 exerts a direct effect on acidocalcisomes, besides its action on the mitochondrial electrochemical potential, causing the disruption of the parasite Ca^{2+} homeostasis.

Materials and methods

Chemicals

Digitonin, EGTA, carbonyl cyanide-*p*-(trifluoromethoxy) phenylhydrazone (FCCP), and nigericin were purchased from Sigma (St. Louis, MO). Fura-2-acetoxymethyl ester (Fura-2-

Fig. 1 Chemical structures of some anti-leishmanial drugs that operate through disruption of the parasite Ca^{2+} homeostasis



AM), acridine orange, and rhodamine 123 were obtained from Molecular Probes (Eugene, OR). Compound SQ109 (Fig. 1) was provided by Otto Geoffroy from Alchem Laboratories Corporation.

Parasites and host cell cultures

L. donovani promastigotes (DD8 strain) were grown in liver infusion tryptose (LIT) medium, pH 7.4, supplemented with 10% fetal bovine serum at 29 °C.

J774 macrophages (lymphoma cells from BALB/c mouse) were employed as host cells and were grown in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal bovine serum at 37 °C, 5% CO_2 .

Effect of SQ109 on the proliferation and viability of *L. donovani* promastigotes

Proliferation was measured as reported previously (Benaim et al. 2006) with some modifications. Parasites (2.5×10^6 parasites/ml) were incubated in LIT medium supplemented with 10% fetal bovine serum containing increasing concentrations of SQ109 at 29 °C. Parasites without any treatment and parasites incubated with DMSO (vehicle) were included as control. The number of parasites in each culture was determined daily by direct counting using a Neubauer chamber.

Effect of SQ109 on macrophages infected with *L. donovani* amastigotes

Amastigote susceptibility assays were performed as reported (Benaim et al. 2012) with some modifications. J774 macrophages were placed simultaneously with *L. donovani* promastigotes over plastic coverslips inside a 24-well plate,

using a ratio of 1:20 macrophages:parasites. After a 24-h incubation to ensure macrophage adhesion and parasite invasion, the wells were washed three times with PBS to remove any non-adherent macrophages and non-internalized parasites. Subsequently, DMEM medium with increasing concentrations of SQ109 was added to the wells and cells were incubated for 48 h, including the appropriate controls. Coverslips were washed with PBS, sealed with methanol, and stained with Giemsa. The percentage of infected cells was determined by light microscopy.

Measure of the intracellular Ca^{2+} concentration of *L. donovani* promastigotes

Intracellular Ca^{2+} concentration was measured as reported previously (Benaim et al. 2006). Briefly, parasites (5×10^5) were washed and resuspended in 500 μl of Tyrode's buffer (137 mM NaCl, 4 mM KCl, 1.5 mM KH_2PO_4 , 8.5 mM Na_2HPO_4 , 11 mM glucose, 1.8 mM CaCl_2 , 0.8 mM MgSO_4 , 20 mM HEPES-NaOH [pH 7.4]). Subsequently, Fura 2-AM (1 μM), probenecid (2.4 mM), and pluronic acid (0.05%) were added and the parasites were incubated under continuous stirring for 2 h at 29 °C at darkness. Parasites were washed twice with the same buffer and loaded into a stirred cuvette. When experiments were performed in the absence of Ca^{2+} , CaCl_2 was omitted from the medium and EGTA was added at 500 μM as a cheating agent, to deplete extracellular Ca^{2+} . Fluorescence measurements were obtained using a Perkin-Elmer spectrofluorimeter LS-55 with a double wavelength excitation beam. Fura 2 is a radiometric indicator for Ca^{2+} which has a peak at 340 nm (when is bound to Ca^{2+}) and another at 380 nm (in the absence of Ca^{2+}), and the emission was recorded at 510 nm.

Determination of the mitochondrial membrane potential ($\Delta\Psi_m$) of *L. donovani* promastigotes

Mitochondrial membrane potential was measured as reported previously (Benaim et al. 2006). Briefly, the fluorescent dye rhodamine 123 was used for the measurement of the effect of SQ109 on the parasite mitochondrial membrane potential ($\Delta\Psi_m$). The dye internalization into the mitochondria is dependent on the magnitude of the $\Delta\Psi_m$. Parasites (5×10^5) were washed and resuspended on loading buffer (130 mM KCl, 1 mM MgCl₂, 2 mM KH₂PO₄, 20 mM Tris-HCl) containing 1% glucose. Subsequently, rhodamine 123 (20 μ M) was added and incubated for 45 min at 29 °C under continuous stirring at darkness. Parasites were washed twice, resuspended in the same buffer, and transferred to a stirred cuvette. Measurements using different concentrations of SQ109 (excitation wavelength [λ_{ext}], 488 nm; emission wavelength [λ_{em}], 530 nm) were carried out in a Hitachi 7000 spectrofluorimeter at 29 °C. The protonophore FCCP (2 μ M) was used as a positive control.

Determination of the effect of SQ109 on acidocalcisomes from *L. donovani* promastigotes

The effect of SQ109 on acidocalcisomes was performed as reported previously (Benaim et al. 2012). Acridine orange was used as fluorescent dye since it internalizes in acidic compartments of the cell. Promastigotes (5×10^5) were washed and resuspended in loading buffer (130 mM KCl, 1 mM MgCl₂, 2 mM KH₂PO₄, 20 mM Tris-HCl) containing 1% glucose. Subsequently, acridine orange (2 μ M) was added and incubated for 15 min at 29 °C under continuous stirring in the dark. Parasites were washed twice, resuspended in the same buffer, and transferred to a stirred cuvette. Measurements using different concentrations of SQ109 (excitation wavelength [λ_{ext}], 488 nm; emission wavelength [λ_{em}], 530 nm) were performed in a Hitachi 7000 spectrofluorimeter at 29 °C. As a positive control, we used nigericin, which is a monovalent ionophore acting as a K⁺/H⁺ exchanger, thus alkalinizing the acidocalcisomes.

Statistical analysis

Half maximal inhibitory concentration (IC₅₀) values were calculated using the GraphPad Prisma 5 Software. All assays were conducted at least in triplicate. The mean and standard deviation were obtained using Microsoft Excel and the statistical significance was calculated using the Student T test.

Results

In order to determine the anti-proliferative effect of SQ109 on promastigotes of *L. donovani* populations, parasites were exposed to different concentrations of SQ109 and were counted daily. The results obtained (Fig. 2) demonstrate that SQ109 inhibits the proliferation of promastigotes of *L. donovani* in a dose-dependent manner, a 100% inhibition of proliferation being observed at a concentration of 5 μ M and an almost complete inhibition of growth at a concentration of 2 μ M. Based on the proliferation plot of promastigotes of *L. donovani* (Fig. 2), a dose-response curve was constructed after 96 h of treatment. The IC₅₀ obtained was 630 ± 61.8 nM.

To evaluate the effect of SQ109 on the percentage of infection of murine macrophages by amastigotes of *L. donovani*, we infected the macrophages with parasites and studied the effect of different concentrations of SQ109. The number of infected cells was counted for each drug concentration (100 macrophages per concentration were counted). Figure 3 shows that SQ109 decreased the number of infected macrophages in a dose-dependent manner, where concentrations greater than 100 nM achieved more than 80% of the effect. The IC₅₀ obtained was 7.17 ± 0.09 nM, demonstrating a strong effect of SQ109 against the intracellular stage of the parasite, the clinically relevant form.

The effect of SQ109 on the intracellular Ca²⁺ concentration of promastigotes of *L. donovani* was determined using Fura 2 as a fluorescence Ca²⁺ indicator. In these experiments, it was found that the addition of SQ109 at a concentration of 10 μ M produces an increase in the 340/380 fluorescence ratio, and this increase is obtained both in the presence (Fig. 4a) and in the absence (Fig. 4b) of Ca²⁺ (EGTA) in the extracellular medium. In addition, the slopes are very similar in both cases.

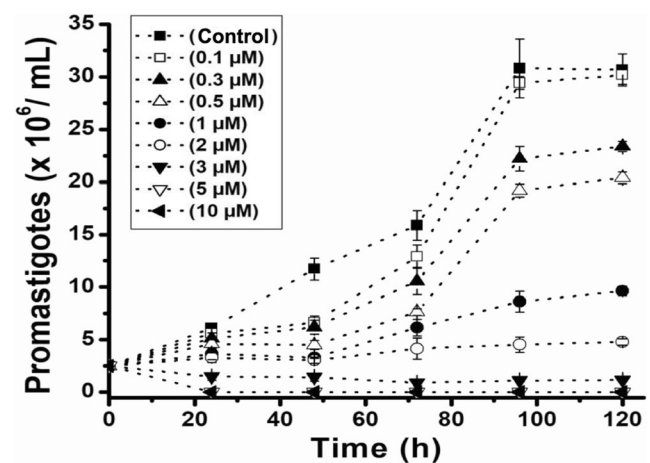


Fig. 2 Effect of SQ109 on *Leishmania donovani* promastigote proliferation. Cultures of *L. donovani* promastigotes were grown in the presence of different concentrations of SQ109 as indicated. Each bar represents the standard deviation of the experimental points of at least three independent experiments

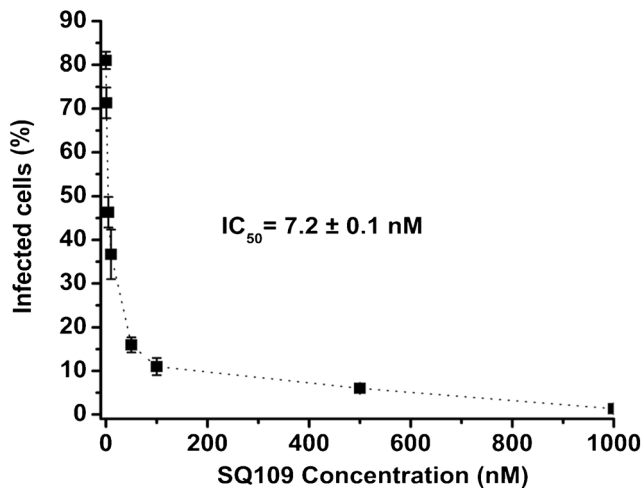


Fig. 3 Effect of SQ109 against intracellular amastigotes of *Leishmania donovani*. The percentage of infected cells (squares) and the IC_{50} on amastigotes were determined 96 h post-treatment. Experiments were carried out in triplicate and each bar represents the standard deviation of the experimental points

These results indicate that the increase in the $[Ca^{2+}]_i$ is consequence of its release from intracellular organelles, probably the mitochondrion and/or the acidocalcisomes.

In order to determine the effect of SQ109 on the mitochondrial electrochemical potential of promastigotes of *L. donovani*, parasites were loaded with the fluorochrome rhodamine 123. Subsequently, changes in the fluorescence of rhodamine 123 were determined in response to the addition of SQ109. An increase in the fluorescence of rhodamine 123 represents the release of the dye as a consequence of a drop in $\Delta\Psi_m$. Figure 5a shows that SQ109 (5 μ M) induced an increase in fluorescence, indicating a collapse of the $\Delta\Psi_m$. Subsequent addition of the protonophore FCCP used as a positive control produced a weak effect. Additionally, addition of FCCP (Fig. 5b) induced a large increase in fluorescence, indicating that the uncoupler indeed dissipated the $\Delta\Psi_m$. Further addition of SQ109 (5 μ M) induced a slight increase in fluorescence (Fig. 5b). The dose-dependent

effect of SQ109 on the mitochondrial electrochemical potential of promastigote from *L. donovani* was studied using increasing concentrations of SQ109, followed by the addition of FCCP. To calculate the percentage of fluorescence, it was taken into account that the addition of the effects of SQ109 and FCCP represent 100% of the response of the system, that is, the complete collapse of the mitochondrial electrochemical potential of promastigotes of *L. donovani*. Figure 5c shows that SQ109 at a concentration of 0.5 μ M produces approximately 30% of the response and at a concentration of 1 and 5 μ M produces a response of 55% and 84%, respectively.

To evaluate the effect of SQ109 on the acidocalcisomes of promastigotes of *L. donovani*, the parasites were loaded with acridine orange, a fluorophore that is internalized in acidic compartments, mainly acidocalcisomes in the case of these parasites. The release of the dye, represented by an increase in the measured fluorescence, is due to the alkalization of the acidocalcisomes. The results obtained show that SQ109 (5 μ M) induced an increase in fluorescence. After addition of nigericin, used as a positive control since this ionophore induces the exchange of H^+ from the acidocalcisome interior for cytoplasmic K^+ , a negligible effect of was observed (Fig. 6a). Conversely, upon addition of nigericin (Fig. 6b), a large increase in fluorescence was produced, resulting in the alkalization of these organelles. Additionally, in Fig. 6b, it can be observed that SQ109 (5 μ M) has a small but significant effect after the addition of nigericin. The same occurs with the other concentrations of SQ109 (not shown), possibly due to the release of acridine orange from other acidic compartments inside the cell.

Different concentrations of SQ109 were tested on acridine orange charged parasites, followed by the addition of nigericin. In Fig. 6c, a dose-dependent effect can be observed. Thus, at a concentration of SQ109 0.5 μ M, 52% of the response was produced, while at a concentration of 1 and 5 μ M, a response of 69% and 90%

Fig. 4 Effect of SQ109 on intracellular Ca^{2+} concentration of *Leishmania donovani*. **a** Effect SQ109 at 10 μ M (arrow) on the intracellular Ca^{2+} concentration of *L. donovani* promastigotes in the presence of 2 mM of extracellular Ca^{2+} . **b** Effect of SQ109 (10 μ M) on the intracellular Ca^{2+} concentration of *L. donovani* promastigotes in absence of extracellular Ca^{2+} (in the presence of 1 mM EGTA)

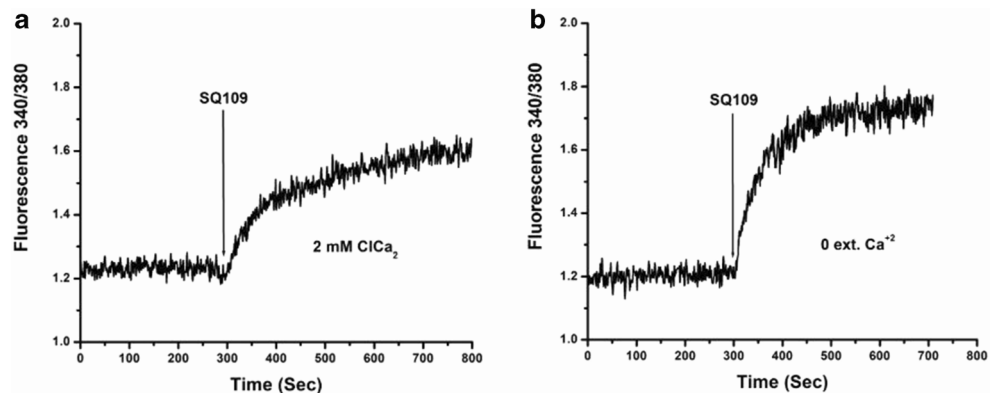


Fig. 5 Effect of SQ109 on the mitochondrial electrochemical potential ($\Delta\Psi_m$) of *Leishmania donovani* promastigotes. **a** SQ109 (5 μM) was added (arrow) to the parasites previously loaded with rhodamine 123, followed by the addition of FCCP (2 μM). **b** FCCP (2 μM) was added (arrow) followed by SQ109 (5 μM). **c** Percentage of rhodamine 123 fluorescence increase with respect to the basal level after addition of different concentrations of SQ109 to *L. donovani* promastigotes. Bars represent the mean \pm SD of three independent experiments. Asterisks represent statistically significant results (** $p \leq 0.01$)

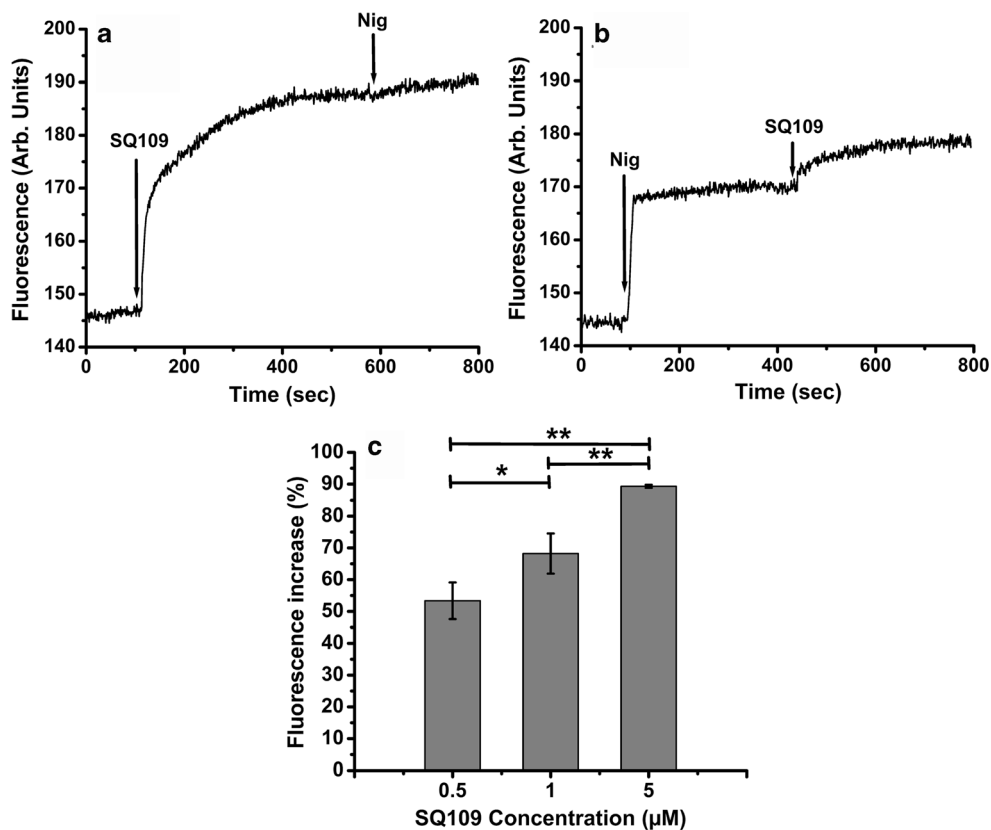
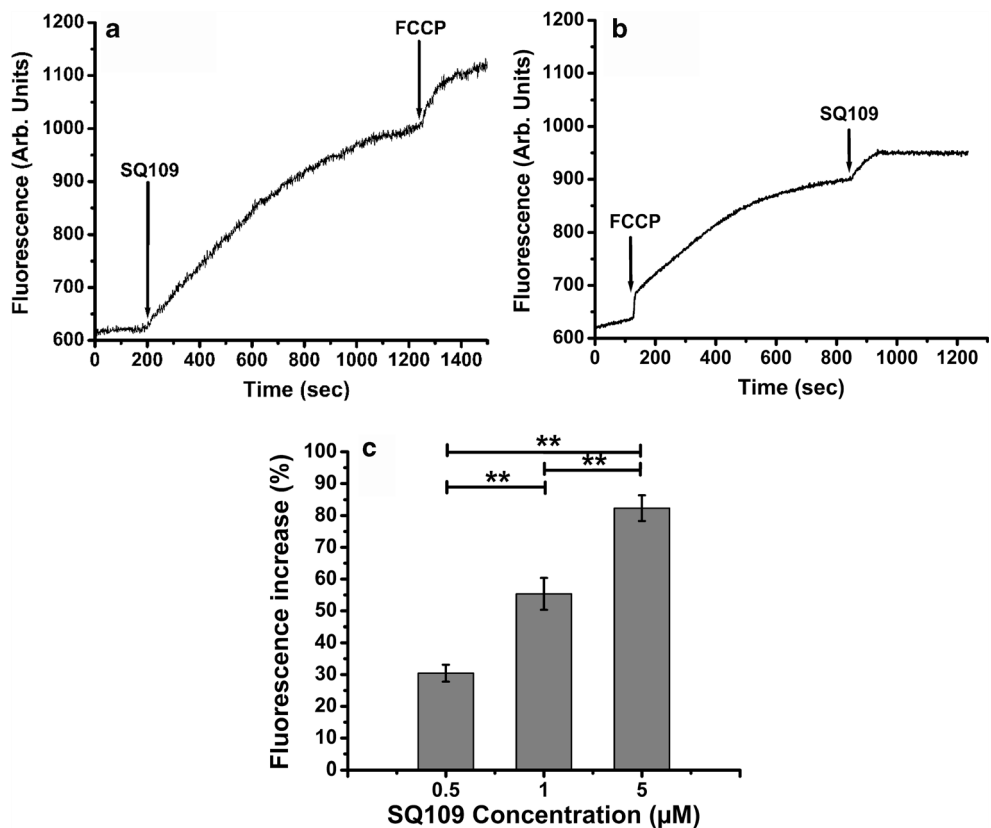


Fig. 6 Effects of SQ109 on the level of acidocalcisome alkalinization in *L. donovani* promastigotes. **a** SQ109 at 5 μM was added (arrow) to parasites loaded with acridine orange, followed by addition of nigericin (2 μM). **b** Addition of nigericin (2 μM) was followed by SQ109 (5 μM). **c** Percentage of acridine orange fluorescence increased with respect to the basal level after addition of different concentration of SQ109 to *L. donovani* promastigotes. Bars represent the mean \pm SD of three independent experiments. Asterisks represent statistically significant results (* $p \leq 0.05$; ** $p \leq 0.01$)



respectively was reached. There are statistically significant differences between all concentrations. These indicate a greater sensitivity of acidocalcisomes to the compound SQ109, when compared to its action on the mitochondria.

Discussion

Different to other leishmaniasis, VL is usually lethal if not treated. As mentioned, SQ109 has a potent inhibitory effect on the growth of *L. donovani* in its promastigote stage, which is of potential interest for the development of hemo-sterilizing agents for blood donations in areas in which there is a high prevalence of VL, as well as for the treatment of both acute and chronic infections with *L. donovani*. On the other hand, the activity of SQ109 on the percentage of infection of amastigotes of *L. donovani* in murine macrophages showed that the compound affects the percentage of infected macrophages in a dose-dependent manner approximately 100-fold more than its effects on promastigotes, a point to take into account given that this is the clinically relevant form of the parasite.

With respect to the extracellular stage, comparing with other reports of repositioned drugs against *L. donovani*, the IC_{50} value for promastigotes treated with SQ109 (630 ± 61.8 nM) is quite promising. This is six times less than the IC_{50} for miltefosine against promastigotes of *L. donovani* (3.74 ± 0.38 μ M) (Prajapati et al. 2013) and very similar to the extracellular IC_{50} of amphotericin B (0.7 ± 0.2 μ M) (Vermeersch et al. 2009). However, amphotericin B commonly has adverse effects if not encapsulated in a lipid-based vehicle and miltefosine has also some limitations since it is teratogenic and apparently induces resistance (Pinto-Martinez et al. 2018a, 2018b). With respect to the intracellular form, the IC_{50} value for amastigotes treated with SQ109 (7.17 ± 0.09 nM) is significantly lower when compared to two approved compounds. Accordingly, the IC_{50} of SQ109 is 614 times smaller than the IC_{50} value for *L. donovani* amastigotes treated with miltefosine (4.3 ± 1.1 μ M) and 57 times lower compared to amphotericin B (0.4 ± 0.2 μ M) (Vermeersch et al. 2009). This marked difference gives SQ109 a greater potential of effectiveness against *L. donovani* compared to traditional treatments, mainly in its amastigote form. This is reinforced by a selectivity index of > 800 , which was calculated by dividing the IC_{50} of SQ109 against murine J774-G8 macrophages (5.8 ± 0.4 μ M) (García-García et al. 2016) by the IC_{50} value obtained during this work for *L. donovani*. The leishmanicidal action of SQ109 on the amastigote form obtained in the present work is even more pronounced than that reported for *L. mexicana* ($IC_{50} \sim 11$ nM) (García-García et al. 2016).

Different fluorometry techniques used in this work showed an increase in the $[Ca^{2+}]_i$ of *Leishmania* parasites following addition of SQ109, compatible with the release of Ca^{2+} from intracellular compartments. Our results suggest that the SQ109-dependent increase in $[Ca^{2+}]_i$ in *L. donovani* is caused partially by its action on the parasite mitochondrion, dissipating the $\Delta\Psi_m$, as in *L. mexicana* (García-García et al. 2016). On the other hand, in the present work, we observe for first time an effect of SQ109 on acidocalcisomes, showing a very rapid alkalinization after addition of this drug. Thus, the elevation of the $[Ca^{2+}]_i$ observed upon addition of this drug is due not only to the collapse of the $\Delta\Psi_m$, but also to its action on acidocalcisomes, also involved in the bioenergetics of this parasite, among other important functions. All these results taken together reinforce the notion that disruption of the parasite $[Ca^{2+}]_i$ regulation could be considered as a rational strategy for the use of drugs against this parasite (Benaim and Garcia 2011). Besides, SQ109 might act directly on the parasite plasma and organelle membranes as revealed by electron microscopy images and also on the squalene synthase, a key enzyme in the synthesis of ergosterol in these parasites, as is the case on the effect reported previously in *T. cruzi* (Veiga-Santos et al. 2015).

It is important to emphasize that there are important differences between cutaneous and visceral species. Among others, the host macrophage population targeted by *Leishmania* also differs between cutaneous and visceral species. Viscerotropic parasites infect Kupffer cells, spleen macrophages, and bone marrow macrophages while cutaneous parasites infect inflammatory monocyte-derived macrophages and dendritic cells (McCall et al. 2013). Also supporting general differences among these parasites, it has been demonstrated that *L. donovani* is more resistant to nitric oxide (NO) and hydrogen peroxide than *Leishmania major* (McCall et al. 2013).

One of the main advantages of SQ109 as a repurposed treatment for leishmaniasis is that this drug has already entered many clinical trials for use in resistant tuberculosis. In fact, in humans, SQ109 has a large volume of distribution and a long half-life (61.1 h per 300 mg orally) (Sacksteder et al. 2012). Based on these results, it can be inferred that the expected concentrations in plasma and internal organs of humans receiving doses of 300 mg per day of SQ109 are located in the range of IC_{50} found here against promastigotes and amastigotes of *L. donovani*, thus making SQ109 a promising drug to fight this disease.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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