AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

Inhibition of *Leishmania mexicana* Growth by the Tuberculosis Drug SQ109

Verónica García-García,[a,b] Eric Oldfield,[c] Gustavo Benaim[a,b]

Instituto de Estudios Avanzados, Caracas, Venezuela[a]; Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela (UCV), Caracas, Venezuela[b]; Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA[c]

We report that the tuberculosis drug SQ109 [*N*-adamantan-2-yl-*N*′-((*E*)-3,7-dimethyl-octa-2,6-dienyl)-ethane-1,2-diamine] has potent activity against the intracellular amastigote form of *Leishmania mexicana* (50% inhibitory concentration [IC_{50}], ~11 nM), with a good selectivity index (>500). It is also active against promastigotes (IC_{50}, ~500 nM) and acts as a protonophore uncoupler, in addition to disrupting Ca^{2+} homeostasis by releasing organelle Ca^{2+} into the cytoplasm, and as such, it is an interesting new leishmaniasis drug hit candidate.

There is a need for new drugs to treat the neglected tropical diseases, in particular, Chagas disease and leishmaniasis. In previous work (1–5), we discovered that the antiarrhythmia drugs amiodarone (Fig. 1, compound 1) and dronedarone (Fig. 1, compound 2) had activity against *Trypanosoma cruzi* as well as *Leishmania mexicana*, the causative agents of Chagas disease and one form of cutaneous leishmaniasis, respectively. In addition, amiodarone was found to have partial *in vivo* activity against *T. cruzi* in mice, which was considerably increased when added in combination with posaconazole (1), and in initial clinical work in humans, it has been used to treat parasitic infections (6, 7). The mechanism of action of compounds 1 and 2 is thought to involve uncoupling activity, with the release of Ca^{2+} from intracellular organelles (acidocalcisomes and mitochondria), as well as inhibition of oxidosqualene synthase and hence, ergosterol biosynthesis. Interestingly, another type of uncoupler, the nitrothiazole nitazoxanide (Fig. 1, compound 3), and its active metabolite, tizoxanide (Fig. 1, compound 4), also have activity against *T. cruzi* and *L. mexicana* (8), and compound 4 has been shown to act, at least in part, as an uncoupler, in *Mycobacterium tuberculosis* (9).

Since we and others recently reported (10–13) that another *M. tuberculosis* drug/drug lead (13–15), SQ109 (Fig. 1, compound 5) [*N*-adamantan-2-yl-*N*′-((*E*)-3,7-dimethyl-octa-2,6-dienyl)-ethane-1,2-diamine], also acted as an uncoupler in *Mycobacterium smegmatis*, we tested it against *T. cruzi*, finding 50% inhibitory concentrations (IC_{50}s) of ~50 nM against trypomastigotes, ~5 μM against epimastigotes, and ~1 μM against amastigotes (13). The amastigote result was disappointing, being less effective than that we found with dronedarone (~1 nM); however, since both amiodarone (Cordarone) and dronedarone (Multaq) come with "black box" warnings, we elected to test SQ109 against *L. mexicana*, since it seemed possible that it might have good activity against this parasitic protozoan, much in the same way as it does against *M. tuberculosis* (and *M. smegmatis*).

We show in Fig. 2A and B the effects of SQ109 on the viability of *L. mexicana* promastigotes and intracellular amastigotes (inside J774 macrophages) and on macrophage viability (Fig. 2C and D). As can be seen in Fig. 2A and B, SQ109 inhibits the viability of *L. mexicana* promastigotes in a dose-dependent manner, and as shown in Fig. 2B, the IC_{50} (after 72 h of treatment) is 0.53 ± 0.06 μM (plus/minus indicates the standard error of the mean for at least three independent experiments). There is little effect on macrophage viability, since the IC_{50} (after 72 h of treatment) is 5.8 ± 0.1 μM (Fig. 2C).

More significantly, in the intracellular assay (Fig. 2D), the 50% inhibitory concentration against amastigotes in infected macrophages (after 48 h of treatment) was 11 ± 0.9 nM, with no observable effect on uninfected macrophages. These results are clearly much more impressive than those we reported earlier with SQ109 in *T. cruzi* and represent a good selectivity index (calculated by IC_{50} of J774 macrophages/IC_{50} of *L. mexicana* amastigotes) of >500. The question then arises, what is the mechanism of action of SQ109 in *L. mexicana*?

As noted above, in earlier work on *M. smegmatis*, we and others (10–12) showed that a major mechanism of action of SQ109 (as well as amiodarone) was on the proton motive force (PMF), with SQ109 collapsing pH gradients, as determined by nuclear magnetic resonance spectroscopy, and electrochemical potentials (in both *M. smegmatis* and *Escherichia coli* membrane vesicles), as determined by fluorescence spectroscopy. Similar results were obtained with amiodarone and dronedarone in *T. cruzi* and *L. mexicana* (1–5). Here, as shown in Fig. 3A, we find that SQ109 collapses the PMF in *L. mexicana* promastigotes, as determined using rhodamine 123. The addition of the known uncoupler FCCP [carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazine] after the addition of SQ109 (5 μM) has no further effect, since the PMF is already collapsed, but if FCCP (2 μM) is added first, there is a partial collapse in the PMF, which is complete after the addition of SQ109 (5 μM) (Fig. 3B). These results are similar to the effects of dronedarone on *L. mexicana* (5). Likewise, we find that SQ109 causes a release of Ca^{2+} from internal Ca^{2+} (Ca^{2+}_i) stored in organelles (Fig. 3C and D). In the presence of 2 mM external Ca^{2+}, the same amount of Ca^{2+} is released by SQ109 (as determined

Received 29 April 2016 **Returned for modification** 23 May 2016
Accepted 19 July 2016

Accepted manuscript posted online 25 July 2016

Citation García-García V, Oldfield E, Benaim G. 2016. Inhibition of *Leishmania mexicana* growth by the tuberculosis drug SQ109. Antimicrob Agents Chemother 60:6386–6389. doi:10.1128/AAC.00945-16.

Address correspondence to Eric Oldfield, eoldfiel@illinois.edu, or Gustavo Benaim, gbenaim@idea.gob.ve.

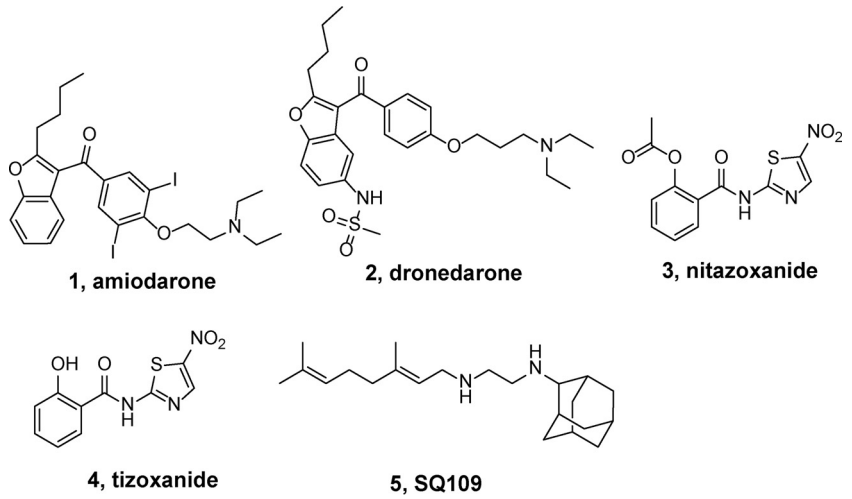

FIG 1 Structures of compounds discussed in the text.

using fura 2) as in the absence of external Ca^{2+} (i.e., in the presence of 8 mM ethylene glycol tetraacetic acid [EGTA], a high-affinity Ca^{2+}-specific chelator), which means that it is not the external Ca^{2+} that is involved in the increase in Ca^{2+}_i. Rather, Ca^{2+} is released from internal stores, mitochondria and acidocalcisomes, again, just as found for *T. cruzi* and *L. mexicana* with amiodarone and dronedarone (1–5). This disruption of Ca^{2+} homeostasis in addition to the effects on the proton motive force (as seen also in mycobacteria [10–12]) are likely to make major contributions to *L. mexicana* cell killing.

Overall, the results we described above are of interest, since we find that the tuberculosis drug SQ109, currently in clinical trials, has potent activity (IC_{50}, 11 ± 0.9 nM) against the clinically relevant amastigote form of *Leishmania mexicana*, inside macro-

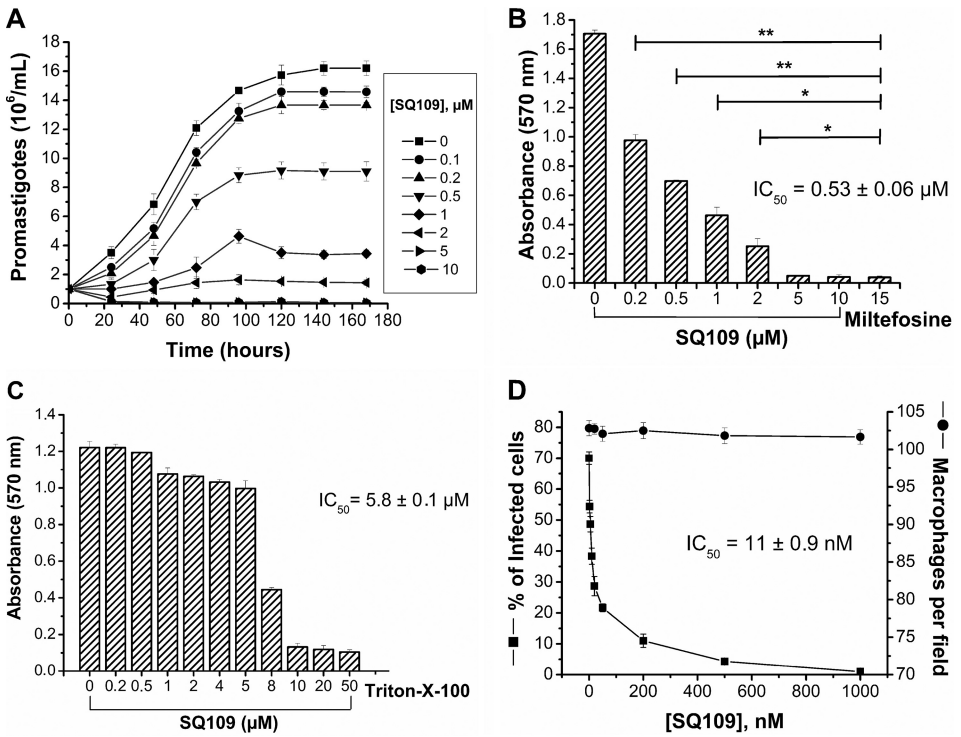

FIG 2 Effects of SQ109 on *L. mexicana* and J774 macrophages. (A) Promastigote growth as a function of time and SQ109 concentration. (B) Effects of SQ109 on the viability of *L. mexicana* promastigotes. The IC_{50} (after 72 h of treatment) is 0.53 ± 0.06 μM. Miltefosine is shown as a positive control. The asterisks represent statistically significant differences, determined using the Student *t* test, $P \leq 0.05$ (*) and $P \leq 0.01$ (**). (C) Effects of SQ109 on J774 macrophage viability; IC_{50} (after 72 h of treatment) is 5.8 ± 0.1 μM. (D) Effects of SQ109 on macrophages infected with *L. mexicana* amastigotes. SQ109 potently inhibits amastigote proliferation, yielding an IC_{50} (after 48 h of treatment) of 11 ± 0.9 nM. The protocols used in panels A and D are the same as those described in reference 5 and for panels B and C in reference 16 (the error bars represent standard deviation for least three independent experiments).

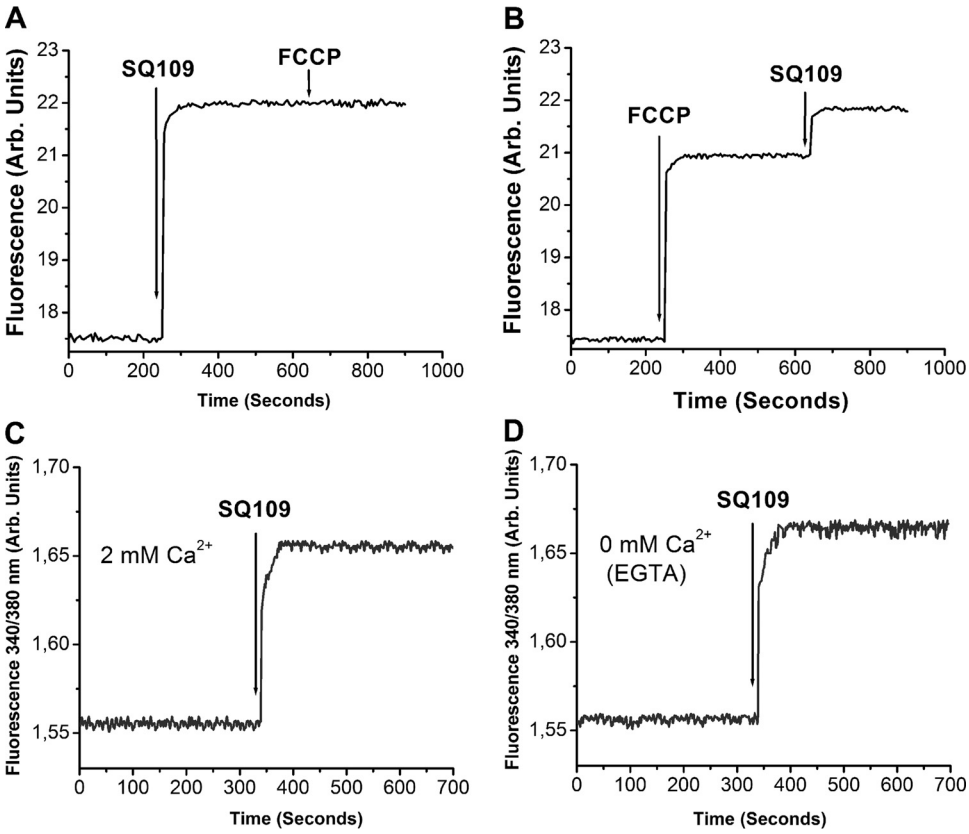

FIG 3 Effects of SQ109 and FCCP on the mitochondrial electrochemical potential and SQ109 on Ca^{2+} flux. (A) SQ109 at 5 μM, followed by 2 μM FCCP using rhodamine 123 as a probe of the electrochemical potential. (B) Same as panel A but FCCP added first and then SQ109. (C) Ca^{2+}_i as determined using the radiometric fluorescent Ca^{2+} indicator fura 2 in the presence of 2 mM external Ca^{2+}. (D) Same as panel C but no external Ca^{2+} (8 mM EGTA). Methods are as described in reference 5.

phages, with a selectivity index of >500. SQ109 appears to act, at least in part, as a protonophore uncoupler (as it does in mycobacteria), in addition to releasing Ca^{2+} from intracellular stores, basically the same mechanism as that found with amiodarone and dronedarone, and as such, it may represent a potential new hit candidate for treating leishmanial diseases.

ACKNOWLEDGMENT

We thank Otto Geoffroy, Alchem Laboratories Corporation, for providing the SQ109.

FUNDING INFORMATION

This work was supported by the Fondo Nacional de Ciencias, Tecnología e Investigación, Venezuela (FONACIT; grant 2011000884 to G.B.), by the Consejo de Desarrollo Científico y Humanístico-Universidad Central de Venezuela (CDCH-UCV; grant PG-03-8728-2013/2 to G.B.), and in part by the U.S. Public Health Service (NIH grants CA158191 and GM065307 to E.O.), a Harriet A. Harlin Professorship (E.O.), and the University of Illinois Foundation/Oldfield Research Fund.

REFERENCES

1. **Benaim G, Sanders JM, Garcia-Marchan Y, Colina C, Lira R, Caldera AR, Payares G, Sanoja C, Burgos JM, Leon-Rossell A, Concepcion JL, Schijman AG, Levin M, Oldfield E, Urbina JA.** 2006. Amiodarone has intrinsic anti-*Trypanosoma cruzi* activity and acts synergistically with posaconazole. J Med Chem **49:**892–899. http://dx.doi.org/10.1021/jm050691f.
2. **Serrano-Martín X, García-Marchan Y, Fernandez A, Rodriguez N, Rojas H, Visbal G, Benaim G.** 2009. Amiodarone destabilizes the intracellular Ca^{2+} homeostasis and the biosynthesis of sterols in *Leishmania mexicana*. Antimicrob Agents Chemother **53:**1403–1410. http://dx.doi.org/10.1128/AAC.01215-08.
3. **Benaim G, Hernandez-Rodriguez V, Mujica-Gonzalez S, Plaza-Rojas L, Silva ML, Parra-Gimenez N, Garcia-Marchan Y, Paniz-Mondolfi A, Uzcanga G.** 2012. *In vitro* anti-*Trypanosoma cruzi* activity of dronedarone, a novel amiodarone derivative with an improved safety profile. Antimicrob Agents Chemother **56:**3720–3725. http://dx.doi.org/10.1128/AAC.00207-12.
4. **Benaim G, Paniz-Mondolfi AE.** 2012. The emerging role of amiodarone and dronedarone in Chagas disease. Nat Rev Cardiol **9:**605–609. http://dx.doi.org/10.1038/nrcardio.2012.108.
5. **Benaim G, Casanova P, Hernandez-Rodriguez V, Mujica-Gonzalez S, Parra-Gimenez N, Plaza-Rojas L, Concepcion JL, Liu Y-L, Oldfield E, Paniz-Mondolfi A, Suarez AI.** 2014. Dronedarone, an amiodarone analog with improved anti-*Leishmania mexicana* efficacy. Antimicrob Agents Chemother **58:**2295–2303. http://dx.doi.org/10.1128/AAC.01240-13.
6. **Paniz-Mondolfi AE, Perez-Alvarez AM, Reyes-Jaimes O, Socorro G, Zerpa O, Slova D, Concepcion JL.** 2008. Concurrent Chagas' disease and borderline disseminated cutaneous leishmaniasis: the role of amiodarone as an antitrypanosomatidae drug. Ther Clin Risk Manag **4:**659–663. http://dx.doi.org/10.2147/TCRM.S2801.
7. **Paniz-Mondolfi AE, Perez-Alvarez AM, Lanza G, Marquez E, Concepcion JL.** 2009. Amiodarone and itraconazole: a rational therapeutic approach for the treatment of chronic Chagas' disease. Chemotherapy **55:**228–233. http://dx.doi.org/10.1159/000219436.
8. **Chan-Bacab MJ, Hernández-Núñez E, Navarrete-Vázquez G.** 2009. Nitazoxanide, tizoxanide and a new analogue [4-nitro-*N*-(5-nitro-1,3-thiazol-2-yl)benzamide; NTB] inhibit the growth of kinetoplastid para-

sites (*Trypanosoma cruzi* and *Leishmania mexicana*) *in vitro*. J Antimicrob Chemother **63:**1292–1293. http://dx.doi.org/10.1093/jac/dkp117.

9. **de Carvalho LPS, Darby CM, Rhee KY, Nathan C.** 2011. Nitazoxanide disrupts membrane potential and intrabacterial pH homeostasis of *Mycobacterium tuberculosis*. ACS Med Chem Lett **2:**849–854. http://dx.doi.org/10.1021/ml200157f.
10. **Li K, Schurig-Briccio LA, Feng X, Upadhyay A, Pujari V, Lechartier B, Fontes FL, Yang H, Rao G, Zhu W, Gulati A, No JH, Cintra G, Bogue S, Liu YL, Molohon K, Orlean P, Mitchell DA, Freitas-Junior L, Ren F, Sun H, Jiang T, Li Y, Guo RT, Cole ST, Gennis RB, Crick DC, Oldfield E.** 2014. Multitarget drug discovery for tuberculosis and other infectious diseases. J Med Chem **57:**3126–3139. http://dx.doi.org/10.1021/jm500131s.
11. **Li W, Upadhyay A, Fontes F, North EJ, Wang Y, Crans DC, Grzegorzewicz AE, Jones V, Franzblau SG, Lee RE, Crick DC, Jackson M.** 2014. Novel insights into the mechanism of inhibition of MmpL3, a target of multiple pharmacophores in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother **58:**6413–6423. http://dx.doi.org/10.1128/AAC.03229-14.
12. **Feng X, Zhu W, Schurig-Briccio LA, Lindert S, Shoen C, Hitchings R, Li J, Wang Y, Baig N, Zhou T, Kim BK, Crick DC, Cynamon M, McCammon JA, Gennis RB, Oldfield E.** 2015. Antiinfectives targeting enzymes and the proton motive force. Proc Natl Acad Sci U S A **112:**E7073–E7082. http://dx.doi.org/10.1073/pnas.1521988112.
13. **Veiga-Santos P, Li K, Lameira L, de Carvalho TMU, Huang G, Galizzi M, Shang N, Li Q, Gonzalez-Pacanowska D, Hernandez-Rodriguez V, Benaim G, Guo RT, Urbina JA, Docampo R, de Souza W, Oldfield E.** 2015. SQ109, a new drug lead for Chagas disease. Antimicrob Agents Chemother **59:**1950–1961. http://dx.doi.org/10.1128/AAC.03972-14.
14. **Heinrich N, Dawson R, du Bois J, Narunsky K, Horwith G, Phipps A, Nacy C, Aarnoutse R, Boeree M, Gillespie S, Venter A, Henne S, Rachow A, Phillips P, Hoelscher M, Diacon AH, Pan African Consortium for the Evaluation of Antituberculosis Antibiotics (PanACEA).** 2015. Early phase evaluation of SQ109 alone and in combination with rifampicin in pulmonary TB patients. J Antimicrob Chemother **70:**1558–1566. http://dx.doi.org/10.1093/jac/dku553.
15. **Sacksteder K, Protopopova M, Barry C, Andries K, Nacy C.** 2012. Discovery and development of SQ109: a new antitubercular drug with a novel mechanism of action. Future Microbiol **7:**823–837. http://dx.doi.org/10.2217/fmb.12.56.
16. **Muelas-Serrano S, Nogal-Ruiz J, Gómez-Barrio A.** 2000. Setting of a colorimetric method to determine the viability of *Trypanosoma cruzi* epimastigotes. Parasitol Res **86:**999–1002. http://dx.doi.org/10.1007/PL00008532.