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ARTICLE

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Microwave-assisted synthesis of 2-styrylquinoline-4-carboxylic acid derivatives to improve the toxic effect against *Leishmania (Leishmania) amazonensis*

Ayelen Luczywo[1] | **Ismael Pretto Sauter**[2] | **Thalita Camêlo da Silva Ferreira**[2] | **Mauro Cortez**[2] | **Gustavo P. Romanelli**[3] | **Gabriel Sathicq**[3] | **Silvia E. Asís**[1]

[1]Facultad de Farmacia y Bioquímica, Departamento de Química Orgánica, Universidad de Buenos Aires, Buenos Aires, Argentina

[2]Laboratório de Imunobiologia da Interação Leishmania-macrófagos, Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil

[3]Centro de Investigación y Desarrollo en Ciencias Aplicadas "Dr. Jorge J. Ronco" (CINDECA-CONICET-CCT-La Plata), Universidad Nacional de La Plata, La Plata, Argentina

Correspondence
Mauro Cortez, Laboratório de Imunobiologia da Interação Leishmania-macrófagos, Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes, 1374, CEP 05508-000 São Paulo, Brazil.
Email: mcortez@usp.br

Silvia E. Asís, Facultad de Farmacia y Bioquímica, Departamento de Química Orgánica, Universidad de Buenos Aires, Junín 956, C1113AAD Buenos Aires, Argentina.
Email: elizabet@ffyb.uba.ar

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The identification of new compounds is urgent to develop safe and efficacious candidates for leishmaniasis treatment, especially from natural products as a potential source of active molecules against neglected tropical parasite diseases. Inspired by the efficacious quinoline alkaloid microbial effects, we have previously reported the synthesis and biological activity of 2-phenylquinoline-4-carboxylic acids and poly-substituted quinolines against parasites. In this work, a series of eighteen 2-styryl-4-quinolinecarboxylic acids were synthesized under microwave irradiation settings obtaining from good to excellent yields (60%-90%), shorter reaction times (2 minutes), and eco-friendly experimental conditions. All these products were evaluated against infective forms of *Leishmania (Leishmania) amazonensis*, such as promastigotes and intracellular amastigotes, based on cytotoxicity assays, including host macrophage infection assays. Compounds **4** and **5** possessing a 2-chloro or 4-chlorostyryl moiety, respectively, were considered the most promising antileishmanial agents due to the parasite killing effect in intracellular forms inside infected macrophages. Thus, our results revealed that the 2-styryl-4-quinolinecarboxylic acid backbone structure was essential for the activity against intracellular pathogens like *L. (L.) amazonensis*.

1 | INTRODUCTION

Leishmaniasis is a neglected vector-transmitted tropical disease, affecting 12 million people around the world, with 350 million at risk of infection in at least 98 countries and territories.[1,2] The disease caused by a protozoan parasites species of the genus *Leishmania*, which is transmitted by sandflies, presents a broad spectrum of clinical manifestations, where three principal ones stand out: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL).[3,4] Known also as kala-azar, VL is fatal if left untreated in over 95% of cases. Most of the human cases occur in Brazil, India, East Africa, and South-East Asia, with an estimated 50 000 to 90 000 new cases occurring worldwide each year, out of which only about 25% to 45% are reported to the World Health Organization (WHO). CL is the most common form of leishmaniasis and causes skin lesions, mainly ulcers, on exposed parts of the body, whereas MCL leads to the partial or total destruction of mucous membrane tissues such as nose, mouth, and throat. About 95% of CL/MCL cases occur in the Americas (mainly in Brazil, Peru, Colombia, and Mexico), the Mediterranean Basin, the Middle East, and Central Asia, with annually estimated 600 000 to 1 million new cases worldwide.[2]

The pharmacological treatment for leishmaniasis is limited, relying primarily on pentavalent antimonials, amphotericin B (under deoxycholate or liposomal formulations), and miltefosine.[5,6] The WHO has made significant efforts by promoting equitable access to health services and essential drug treatment. Significant achievements include reducing the price of two of the five existing medicines for VL by 90% for liposomal amphotericin B and by 60% for meglumine antimoniate, and a new agreement until 2021 for the donation of 445 000 vials of liposomal amphotericin B injection to treat more than 50 000 VL cases in eligible endemic countries.[2] However, these few chemotherapeutic options are mostly unsatisfactory not only by the high costs but also due to low efficacy, poor safety, the emergence of parasite resistance, among other factors related to treatment (such as administration route or length of treatment) that are unsuitable for the socioeconomic reality of affected populations.[6–11] Therefore, the identification of new compounds is urgent to develop safe and efficacious candidates for leishmaniasis treatment, especially from natural products as a potential source of active molecules against neglected tropical parasite diseases.[3] In this context, new and established pharmacophores are being identified based on synthetic and natural product chemistry.

Leishmaniasis chemotherapy has been historically associated with natural products, such as amphotericin B extracted from the filamentous bacteria *Streptomyces nodosus*, and paromomycin obtained from *Streptomyces rimosus*.[12] Moreover, reports identifying natural products with antileishmanial activity are always frequent. A series of 2-substituted quinolines isolated from *Galipea longiflora* K. Krause (family Rutaceae), a Bolivian tree traditionally used to cure ulcerations of CL by the native Chimanes tribe, was first reported by Fournet et al.[13] Pure quinoline alkaloids, such as 2-*n*-propylquinoline, 2-*n*-pentylquinoline, 4-methoxy-2-phenylquinoline, and 2-(3,4-methylenedioxyphenylethyl)-quinoline, demonstrated promising activity against *Leishmania donovani* (species related to VL) in vitro, and activity by oral route in the in vivo evaluation.[14] This discovery encouraged other authors to develop C2-substituted quinolines as antileishmanial agents.[15,16] Noteworthy, the synthesis of diverse C2-aryl quinoline derivatives with activity against *Leishmania (Vianna) braziliensis* (species related to CL and MCL), and their possible mechanism of action were reported.[17]

In addition to their recognized activity as antimalarials, many synthetic quinoline derivatives are antibacterial, antifungal, antitumor,[18] and antimycobacterial agents.[19,20] We have previously reported the microwave-assisted synthesis of substituted 2-phenylquinoline-4-carboxylic acids[21] and poly-substituted quinolines,[22] both series showing antiparasitic activities. Among them, we found a hit compound with antimalarial and antichagasic (Chagas diseases caused by the protozoan *Trypanosoma cruzi*) activities and four derivatives with moderate activity against *Leishmania (Leishmania) infantum chagasi* and *L. donovani* (Figure 1). Furthermore, 2-methyl and 2-aryl-4-quinolinecarboxylic acids[23] and 4-amino-2-styrylquinolines[24] with antileishmanial activity were recently reported. All these findings prompted us to investigate the influence on the antileishmanial activity of the vinyl-bridge insertion between the 2-aryl ring and the quinoline core of these new 4-quinolinecarboxylic acids.

2 | RESULTS AND DISCUSSION

2.1 | Synthesis and characterization of 2-styryl-4-quinolinecarboxylic acid

Herein, we present the microwave-assisted reaction of 2-methyl-4-quinolinecarboxylic acid (*i*) and arylaldehydes (*ii*) under trifluoroacetic acid (TFA) catalysis (Scheme 1), to obtain eighteen 2-styryl-4-quinolinecarboxylic acids (*iii*, **1-18**). All these products were evaluated against promastigotes and intracellular amastigotes of *L. (L.) amazonensis*, based on cytotoxicity assays, including host macrophages.

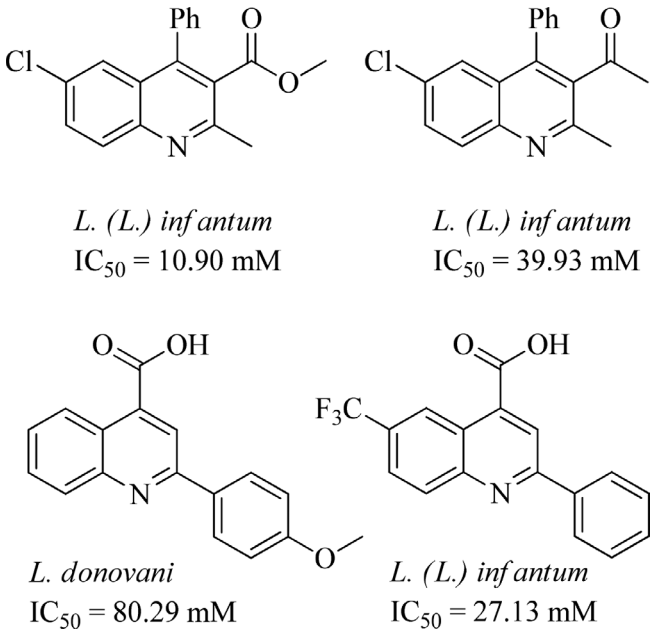
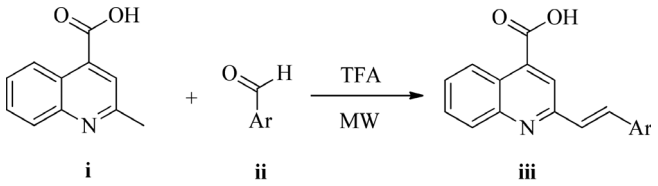


FIGURE 1 2-methyl and 2-aryl-4-quinolinecarboxylic acids (two compounds each) previously shown to possess in vitro antileishmanial activity



iii:
1 Ar = 2-$NO_2C_6H_4$
2 Ar = 3-$NO_2C_6H_4$
3 Ar = 4-$NO_2C_6H_4$
4 Ar = 2-ClC_6H_4
5 Ar = 4-ClC_6H_4
6 Ar = 4-OH-3-$OCH_3C_6H_3$
7 Ar = 3,4,5-*tri*-$OCH_3C_6H_2$
8 Ar = 3,5-*di*-FC_6H_3
9 Ar = 3,4-*di*-FC_6H_3
10 Ar = 2,3-*di*-FC_6H_3
11 Ar = 2,6-*di*-FC_6H_3
12 Ar = 2,5-*di*-FC_6H_3
13 Ar = 2-FC_6H_4
14 Ar = C_6H_5
15 Ar = 3,4-$OCH_2OC_6H_3$
16 Ar = 1-Naph
17 Ar = 6-OCH_3-2-Naph
18 Ar = 4-Br-3,5-*di*-$OCH_3C_6H_2$

SCHEME 1 Microwave-assisted (MW) synthesis from 2-methyl-4-quinolinecarboxylic acid (*i*) and arylaldehydes (*ii*) under trifluoroacetic acid (TFA) catalysis, to obtain 2-arylvinyl-4-quinolinecarboxylic acids (*iii*, **1-18** derivatives)

The starting material (*i*) was prepared employing the Pfitzinger reaction from isatin and ketone,[25] then it was subjected to Knoevenagel condensation to provide the 2-styryl-4-quinolinecarboxylic acids (*iii*) in good to excellent yields (60%-90%) and short reaction times (2 minutes). The ^{1}H NMR spectra showed that the compounds (*iii*) are *E* stereoisomers owing to the value of 16.0 to 16.6 Hz for the double bond hydrogen atoms coupling constant *J*. Moreover, the IR spectra contained absorption bands at 960 to 968 cm^{-1}, typical of stretch vibrations of the CH=CH bond in the *trans* form.[26,27] Derivatives **1** to **3** and **13** were prepared under thermal heating in acetic anhydride,[26,27] whereas compound **6** was obtained from isatin.[28] In the literature, only their melting point values and the ^{1}H NMR spectrum for compound **13** were found.[27] In this work, all the yields are higher than the previously reported values, including **5**, **7**, and **14** to **17**.[9,25] To evaluate the method, the EcoScale Calculator Index was determined.[29] This procedure receives a significantly excellent score of 77 (score from 0 to 100) based on economic and ecological parameters.

Most of the selected arylaldehydes (*ii*) possess systematic changes at positions 2 to 5 of the phenyl-ring to determine the effect of substitution with atoms or moieties, bearing different electronic, hydrophobic, and steric properties on antileishmanial potency and cytotoxicity. In this way, 2-fluoro, 2-nitro, 2-chloro, 4-methoxyphenyl groups, and their position isomers were the main targeted compounds.[21,30] The octanol-water partition coefficient (logP), topological polar surface area (TPSA), the number of hydrogen-bond donors (HBD) and acceptors (HBA), and the number of rotatable bonds for all synthesized compounds (*iii*) were estimated using the online prediction tools from the Molinspiration server.[31]

2.2 | In vitro activity against *L. (L.) amazonensis* and bone marrow macrophages

The inhibitory effect of the different derivative compounds on *L. (L.) amazonensis* proliferation (strain IFLA/BR/67/PH8) was in vitro assayed using the proliferative biological stage promastigote in culture media. The inhibitory concentration of parasite growth was determined by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay[32] after 24, 48, and 72 hours of the proliferation assays in the presence of the compounds, and analyzed statistically by Student *t* test in Prism 5.0 software. All compounds were tested at 29.3 μM, single dose (IC_{50}, previously determined), and each test was carried out in duplicate in two independent experiments. The treatment with compounds **1** to **6** and **14** decreased parasite viability (Figure 2), so they were selected for further investigation of their inhibitory activity in the intracellular parasite proliferative-stage amastigotes, by a biological model previously reported.[33,34]

To perform the assay of the derivatives inhibitory activity against amastigotes, bone marrow macrophages (BMMs) were infected with promastigotes for 2 hours, washed, and incubated in media for 24 hours to obtain

intracellular amastigotes. After incubation, amastigotes-infected BMMs were treated with different derivatives. A 33% to 40% decrease in the number of parasites per cell was observed in compounds **2**, **4**, and **5** (Figure 3A), although the compounds did not affect the percentage of infected cells (Figure 3B). In the representative images of amastigote-infected BMMs (Figure 4), in the untreated sample (Figure 4A) it is possible to visualize two or more parasites inside of the rounded vacuoles (parasitophorous vacuoles, PV) in the BMMs, similarly to the derivatives without effects (compounds **1**, Figure 4C; **3**, Figure 4E; **6**, Figure 4H; and **14**, Figure 4I) whereas in the treatment with inhibitory effect (compounds **2**, Figure 4D; **4**, Figure 4F; and **5**, Figure 4G) individual amastigotes were visualized inside PV (at similar for the inhibitory control such as amphotericin, Figure 4B). From these results, compounds **4** and **5** were considered the most promising antileishmanial agents.

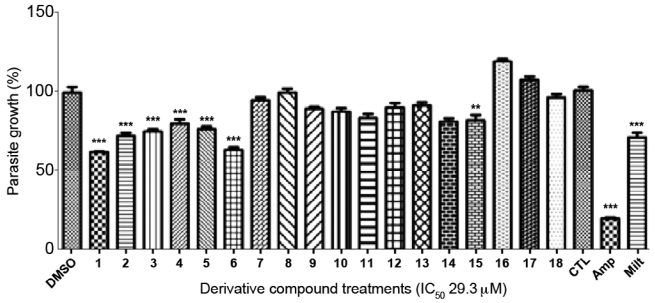


FIGURE 2 Inhibitory effect of 2-arylvinyl-4-quinolinecarboxylic acids derivatives on the proliferation of *L. (L.) amazonensis* promastigotes (29.3 μM, single dose). Amp, amphotericin B; CTL, control sample; DMSO, dimethyl sulfoxide; Milt, Miltefosine. *P*-value: ***$P < .001$; **P .001-.01

2.3 | SAR analysis, cytotoxicity assay, and possible target enzyme

The molecular properties of the active compounds **1** to **6** and **14** were calculated (Table 1). Strikingly, it is worthy to note that only the Log *P* values for the active compounds **4** and **5** (*) exceed Lipinski's rule of five,[35] the other derivative compounds falling within the rule. Lipinski's rule states that, in general, an orally active drug has no more than one violation of the criteria. Moreover, 3D molecular structures were created using Molinspiration Galaxy 3D generator and were examined in various display modes, including visualization of surface properties, such as molecular lipophilicity potential (MLP) and polar surface area (PSA). Compounds **4**, **5**, and **14** exhibited similar MLP and shape, where the (substituted) phenyl moiety is perpendicular to the quinoline-ring. For compounds **1**, **2**, and **6**, the phenyl significantly approaches the quinoline plane and exhibits a hydrophilic region (Figure 5).

When the effects of 2-arylvinyl-4-quinolinecarboxylic acid derivatives on their proliferative (growth%) inhibition activity were compared in both biological forms of *L. (L.) amazonensis*, derivative compounds **1** and **6**, which showed the best inhibitory results on promastigotes, lost any effect on the intracellular amastigotes (as well as compound **14**). In contrast, derivative compounds **2**, **4**, and **5** kept inhibiting the intracellular stage. This information is critical since all these derivative molecules need to cross several membranes (host plasmatic membrane, PV membrane, and parasite surface membrane) until reaching the parasite inside the host cell. It could mean that derivatives **1**, **6**, and **14** were incapable of penetrating the membrane (**1** and **6** possess lower Log

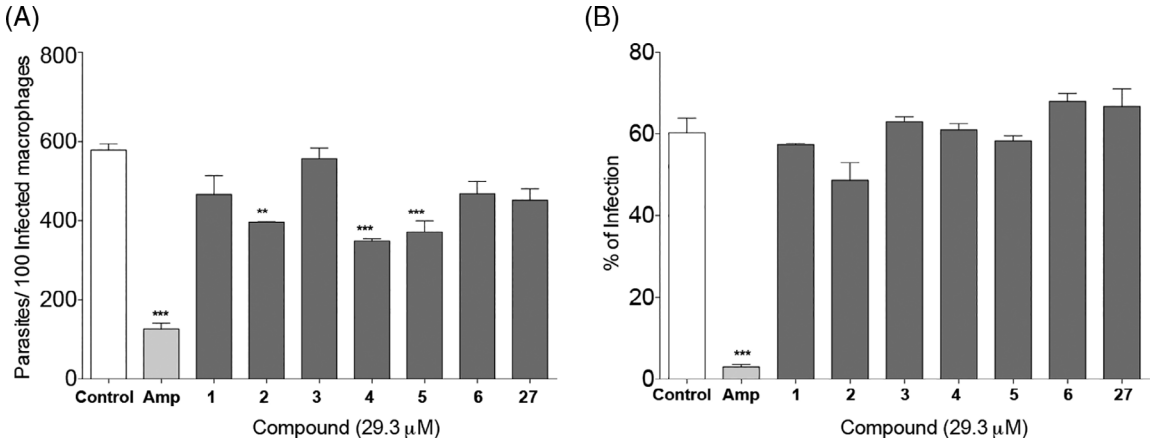


FIGURE 3 Inhibitory effect of 2-arylvinyl-4-quinolinecarboxylic acid derivatives on the proliferation of *L. (L.) amazonensis* intracellular amastigotes (29.3 μM, single dose). Bone marrow macrophage (BMM)-containing intracellular amastigotes and treated with the compounds **1** to **6**, and **14**. A, The number of intracellular parasites per 100 infected BMMs. B, Percentage of amastigotes infection. Control: untreated sample. Amp, amphotericin B. *P*-value: ***$P < .001$; **P .001-.01

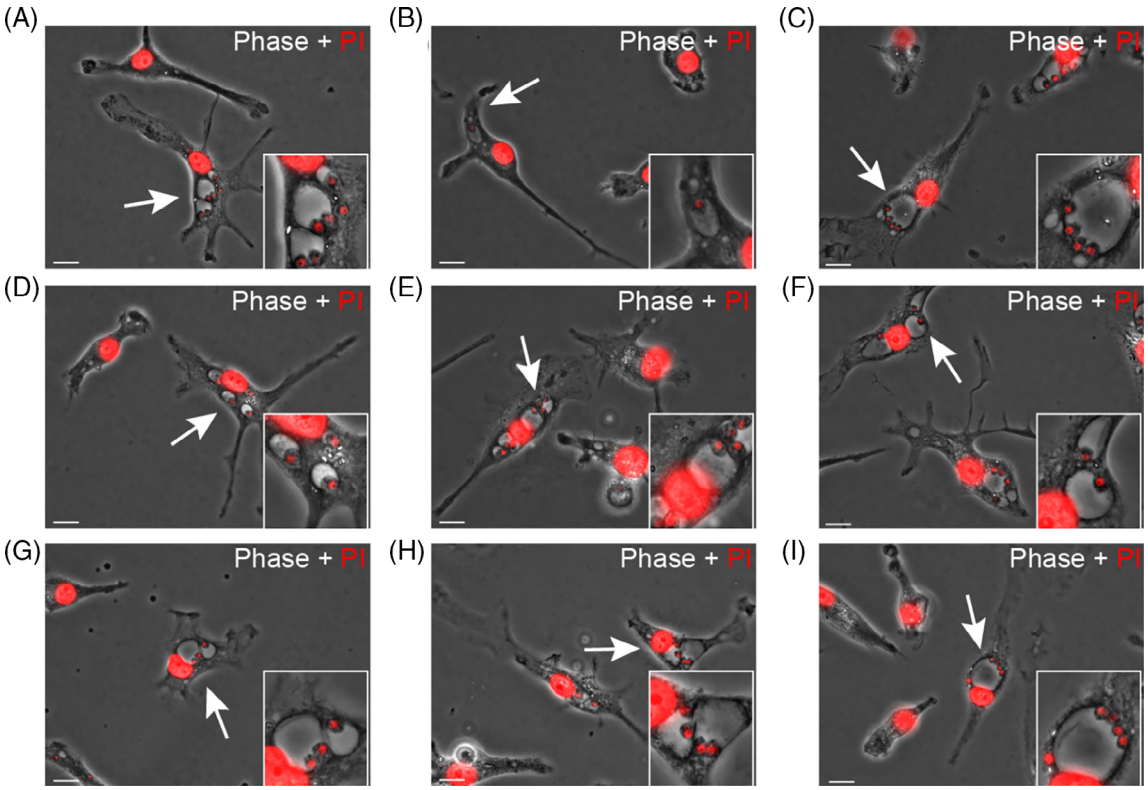


FIGURE 4 Representative images of amastigote-infected bone marrow macrophages (BMMs) untreated (A, Control) or treated as indicated (B, amphotericin B; C, Compound **1**; D, Compound **2**; E, Compound **3**; F, Compound **4**; G, Compound **5**; H, Compound **6**; I, Compound **14**). After incubation, the samples were processed by fluorescence staining to recognize the nuclei of macrophages and parasites (PI, propidium iodide), including an overlay of the contrast phase (phase). Arrows indicate the selected crop for zooming (at the bottom left of the image). Scale bar: 10 μm [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Molecular properties of the active products (*iii*) **1** to **6**, and **14** calculated from Molinspiration server

iii	LogP[a]	TPSA[b]	Number of atoms[c]	MW[d]	Number of ON[e]	Number of OHNH[f]	Ro5[g]
1	4.31	96.02	24	320.30	6	1	0
2	4.52	96.02	24	320.30	6	1	0
3	4.54	96.02	24	320.30	6	1	0
4	5.03*	50.19	22	309.75	3	1	1
5	5.26*	50.19	22	309.75	3	1	1
6	3.92	79.65	24	321.33	5	2	0
14	4.58	50.19	21	275.30	3	1	0

[a]LogP octanol-water partition coefficient;
[b]TPSA, topological polar surface area;
[c]Number of nonhydrogen atoms;
[d]Molecular weight;
[e]Number of hydrogen-bond acceptors (O and N atoms);
[f]Number of hydrogen-bond donors (OH and NH groups);
[g]Number of rule of five violations.
*LogP outside Lipinski's rule of five.

P values compared to the others), or these compounds could be unstable and easily degraded by host factors (acidic intravacuolar environment, degradation by host enzymes, among others). Although these synthetic compounds seem to be quite stable,[25] differences in the final target molecules of the derivative compounds, perhaps in

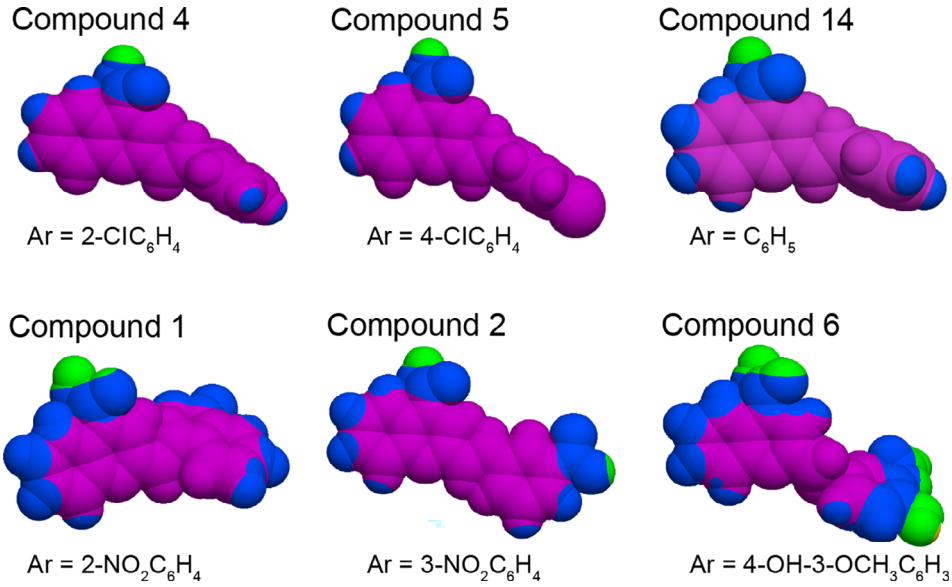


FIGURE 5 Visualization of molecular lipophilicity potential (MLP) for representative 3D molecular structures, to see which parts of the surface are hydrophobic (encoded by violet and blue colors) and which hydrophilic (orange and red) [Colour figure can be viewed at wileyonlinelibrary.com]

the presence or quantities, might differ in the different biological stages, an important feature when comparing in the *Leishmania* spp. drug discovery field.[36,37] Because the more suitable biological form to test new compounds against *Leishmania* spp. is amastigotes,[38,39] derivative compounds **4** and **5** were found to be promising as antileishmanial molecules, according to this SAR analysis.

A further cytotoxicity assay was performed, including some compounds in RAW cells, a macrophage-like cell linage, due to the impossibility to work with mice. As visualized in Figure S1, similar results of viability were obtained with trypan blue staining assay comparing to the control and amphotericin B, for 72 hours. The compounds **5**, **6**, and **14** with leishmanicidal effect are not toxic for the host cells. However, MTT assays showed an effect with the compound **2**, which could be a metabolic effect in macrophages owing to the nitro group.

As a possible candidate affected by 2-arylvinyl-4-quinolinecarboxylic acid derivatives, the flavoenzyme dihydroorotate dehydrogenase (DHODH) is a validated target for the inhibitory effect against *Leishmania* spp., as well as an antiparasite drug in the research field of new drug discovery.[40] DHODHs catalyze the stereoselective oxidation of (*S*)-dihydroorotate (DHO) to orotate (ORO) in the fourth reaction of the de novo pyrimidine biosynthetic pathway, been divided into Class 1 and Class 2 according to sequence similarity, subcellular location, and preference for the substrate.[41] Class 1 DHODHs are cytosolic enzymes, present in *Leishmania* genus and *T. cruzi*, with a few examples of specific inhibitors being mentioned in the literature.[42,43]

Noteworthy, *L. donovani* DHODH has explicitly been proposed as a target for 2-aryl-4-quinolinecarboxylic acids.[23] By aligning the 3D structures of *T. cruzi*, *L. major*, and human DHODH active sites, it can be seen that, despite the substitution of some residues, the profile of interactions for molecular recognition is highly conserved between the two classes.[44] Furthermore, human DHODH inhibitors included brequinar (synthetic 4-quinolinecarboxylic acid) and several series of 4-quinolinecarboxylic acid derivatives,[45] also with antiviral activity,[46] strongly suggesting the active compounds **4** and **5** as *Leishmania* DHODH inhibitors, based on these reported data.

3 | CONCLUSION

Eighteen (*E*) 2-styryl-4-quinolinecarboxylic acids were prepared in good to excellent yields, short reaction times, and simple work-up procedure under eco-friendly conditions. Seven of these derivatives are novel structures and for the remaining known compounds, this work provides additional spectroscopic data as well as an improved synthetic method with higher yields than reported. It was shown that the 2-styryl-4-quinolinecarboxylic acid backbone is essential for the activity against *L. (L.) amazonensis*. The compounds **5**, **6**, and **14** with leishmanicidal effect are not toxic for the host cells. The SAR studies indicate that chlorine atoms attached at the 2-styryl moiety increase such activity, for this reason, compounds **4** and **5** are attractive candidates for hit-to-lead development, maybe as potential DHODH inhibitors.

4 | EXPERIMENTAL SECTION

4.1 | General

The structures of the synthesized compounds were established through their ^{1}H and ^{13}C-NMR, MS, and IR spectra. Melting points were determined in a capillary Electrothermal 9100 SERIES-Digital apparatus and are uncorrected. ^{1}H and ^{13}C-NMR spectra were obtained using a Bruker 600 spectrometer. The operating frequencies for protons and carbons were 600 and 151 MHz, respectively. The chemical shifts (δ) were given in ppm. IR spectra were recorded on an FT Bruker from KBr disks. Mass spectra were measured on MS/DSQ II Thermo Scientific DPC. Elemental analysis data for synthesized compounds were consistent with calculated values. Analytical time of reaction completion (TLCs) was performed on DC-Alufolien Kieselgel 60 F_{254} Merck. Microwave-assisted reactions were carried out in a Glass vial G10, Anton Paar Monowave Series (Serial Number: 81920884, Instrument Software Version: 4.10.9376.7).

4.1.1 | General procedure for the synthesis of 2-arylvinyl-4-quinolinecarboxylic acids *iii* 1 to 18

A neat mixture of 0.25 mmol (0.05 g) of 2-methyl-4-quinolinecarboxylic acid and 0.25 mmol of the corresponding arylaldehyde with 0.1 mL TFA was subjected to MW irradiation at 170°C and 850 W. The mixture was cooled to room temperature to give a solid product which was then triturated from EtOH; TLC is 2 minutes.

4.1.2 | (*E*)-2-(2-nitrostyryl)quinoline-4-carboxylic acid 1

Yield 77%, pale yellow powder, mp > 300°C (lit. 293°C-294°C).[26] ^{1}H NMR (600 MHz, DMSO-d_6) δ 14.04 (s, 1H, H24), 8.67 (d, J = 8.2 Hz, 1H, H3), 8.24 (s, 1H, H9), 8.16 (d, J = 16.2 Hz, 1H, H12), 8.11 (d, J = 8.01 Hz, 1H, H6), 8.10 (t, J = 7.1 Hz, 1H, H16), 8.07 (dd, J = 8.1; 0.9 Hz, 1H,H17), 7.85 (td, J = 8.3; 1.1 Hz, 1H, H15), 7.82 (t, J = 7.5 Hz, 1H, H1), 7.71 (td, J = 8.2; 1.1 Hz, 1H, H2), 7.64 (td, J = 8.3; 0.9 Hz, 1H, H14), 7.63 (d, J = 16.01 Hz, 1H, H11). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.9, 154.9, 148.9, 148.8, 137.6, 134.1, 132.9, 131.4, 130.7, 130.1, 130.0, 129.6, 129.1, 128.4, 125.9, 125.1, 124.3, 121.7. IR (cm^{-1}): ν 3469, 3063, 3032, 1734, 1593, 1523, 1375, 969, 733. Chemical Formula: $C_{18}H_{12}N_2O_4$.

4.1.3 | (*E*)-2-(3-nitrostyryl)quinoline-4-carboxylic acid 2

Yield 86%, pale yellow powder, mp > 320°C (lit. 316°C-317°C).[26] ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.66 (d, J = 8.5 Hz, 1H, H3), 8.62 (s, 1H, H18), 8.31 (s, 1H, H9), 8.28 (d, J = 7.8 Hz, 1H, H6), 8.20 (dd, J = 8.1; 1.6 Hz, 1H, H16), 8.11 (d, J = 8.4 Hz, 1H, H14), 8.08 (d, J = 16.4 Hz, 1H, H12), 7.85 (t, J = 7.6 Hz, 1H, H15), 7.81 (d, J = 16.4 Hz, 1H, H11), 7.75 (t, J = 7.9 Hz, 1H, H1), 7.69 (t, J = 7.6 Hz, 1H, H2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.0, 155.3, 148.9, 148.9, 138.5, 133.8, 132.9, 131.3, 130.8, 130.65, 129.9, 128.2, 126.0, 124.2, 123.6, 122.3, 121.3. IR (cm^{-1}): ν 3438, 3064, 1734, 1593, 1530, 1367, 955, 734. Chemical Formula: $C_{18}H_{12}N_2O_4$.

4.1.4 | (*E*)-2-(4-nitrostyryl)quinoline-4-carboxylic acid 3

Yield 89%, pale yellow powder, mp > 320°C (lit. 324°C-325°C).[26] ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.18 (s, 1H, H24), 8.66 (d, J = 8.5 Hz, 1H, H3), 8.43 (d, J = 8.6 Hz, 1H), 8.34 (s, 1H, H9), 8.29 (d, J = 8.71 Hz, 1H), 8.15 (d, J = 16.6 Hz, 1H, H12), 8.09 to 8.04 (m, 3H), 7.86 (dt, Jo = 8.2, Jp = 0.7 Hz, 1H, H1), 7.82 (d, J = 16.4 Hz, 1H, H11), 7.71 (dt, Jo = 8.0, Jp = 0.7 Hz, 1H, H2). ^{13}C NMR (151 MHz, DMSO-d_6) δ 192.8, 167.9, 155.0, 148.7, 147.5, 143.3, 140.5, 137.7, 133.1, 131.1, 130.9, 129.8, 128.8, 128.5, 126.0, 124.7, 124.5, 124.2, 121.5. IR (cm^{-1}): ν 3438, 3064, 1734, 1593, 1530, 1367, 955, 734. MS: 320.1 (M^+). Chemical Formula: $C_{18}H_{12}N_2O_4$.

4.1.5 | (*E*)-2-(2-chlorostyryl)quinoline-4-carboxylic acid 4

Yield 73%, pale yellow powder, mp 312.4°C-312.6°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.66 (d, J = 8.5 Hz, 1H, H3), 8.20 (s, 1H, H9), 8.18 (d, J = 16.1 Hz, 1H, H12), 8.12 (d, J = 8.4 Hz, 1H, H6), 8.03 (dd, J = 7.6, 1.3 Hz, 1H, H17), 7.83 (dt, J = 8.1, 1.1 Hz, 1H, H2), 7.69 (dt, J = 8.1, 1.1 Hz, 1H, H1), 7.66 (d, J = 16.1 Hz, 1H, H11), 7.56 (dd, J = 7.8,0.9 Hz, 1H, H14), 7.44 (dt, J = 7.2,1.1 Hz, 1H, H15), 7.40 (td, J = 7.6, 1.5 Hz, 1H, H16). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.9, 155.1, 148.9, 137.5, 134.2, 133.5, 131.3, 130.8, 130.7, 130.4, 130.02, 129.9, 128.2, 128.1, 128.0, 125.9, 124.2, 122.0. IR (cm^{-1}): ν 3485, 3050, 1734, 1593, 1390, 953, 875. MS: 309.8 (M^+). Calcd. Analysis for $C_{18}H_{12}ClNO_2$: C, 69.80; H, 3.90; N, 4.52. Found: C, 69.72; H, 4.05; N, 4. 49.

4.1.6 | (*E*)-2-(4-hydroxy-3-methoxystyryl) quinoline-4-carboxylic acid 6

Yield 90%, orange powder, mp 314°C-316°C (lit. 282°C-283°C).[28] ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.91 (s, 1H, H22), 8.61 (dd, J = 8.5; 0.6 Hz, 1H, H3), 8.21 (s, 1H, H9), 8.05 (d, J = 8.3 Hz, 1H, H6), 7.82 (d, J = 16.3 Hz, 1H, H12), 7.80 (td, J = 8.3; 1.2 Hz, 1H, H1), 7.65 (td, J = 8.2; 1.2 Hz, 1H, H2), 7.41 (d, J = 16.2 Hz, 1H, H11), 7.39 (d, J = 2.0 Hz, 1H, H18), 7.18 (dd, J = 8.2;2.0 Hz, 1H, H14), 6.85 (d, J = 8.03 Hz, 1H, H15), 3.87 (s, 3H, OCH3). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.1, 156.3, 148.5, 148.4, 137.1, 135.9, 130.5, 129.5, 128.1, 127.5, 125.9, 125.3, 123.7, 122.1, 121.0, 120.9, 116.1, 111.1, 56.1. IR (cm^{-1}): ν 3453, 3078, 2984, 2945, 1641, 1594, 1390, 1281, 1345, 954, 875. Chemical Formula: $C_{19}H_{15}NO_4$.

4.1.7 | (*E*)-2-(3,5-*di*fluorostyryl) quinoline-4-carboxylic acid 8

Yield 82%, yellow powder, mp 319.3°C-319.9°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.66 (d, J = 8.5 Hz, 1H, H3), 8.20 (s, 1H, H9), 8.17 (d, J = 16.1 Hz, 1H, H12), 8.12 (d, J = 8.5 Hz, 1H, H6), 8.03 (d, J = 7.7 Hz, 1H, H14), 7.83 (t, J = 7.6, 1H, H2), 7.69 (td, J = 8.5, 2.8 Hz, 1H, H1), 7.65 (d, J = 16.1 Hz, 1H, H11), 7.55 (d, J = 8.9 Hz, 1H, H18), 7.46 to 7.40 (m, 1H, H16). ^{13}C NMR (151 MHz, DMSO-d_6) δ (151 MHz, DMSO) δ 167.9, 164.0, 162.3, 155.2, 148.9, 140.6, 140.5, 137.3, 132.9, 131.3, 130.7, 129.9, 128.3, 125.9, 124.2, 121.4, 110.7, 104.5, 104.3, 104.2. IR (cm^{-1}): ν 3438, 3078, 3031, 1640, 1593, 1328, 1125, 984, 859. Calcd. Analysis for $C_{18}H_{11}F_2NO_2$: C, 69.45; H, 3.56; N, 4.50. Found: C, 69.38; H, 3.63; N, 4.43.

4.1.8 | (*E*)-2-(3,4-*di*fluorostyryl) quinoline-4-carboxylic acid 9

Yield 84%, yellow powder, mp 309.0°C-309.6°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.64 (d, J = 7.9 Hz, 1H, H3), 8.23 (s, 1H, H9), 8.08 (d, J = 8.1 Hz, 1H, H6), 7.91 (ddd, J = 7.9, 1.7 Hz, 1H, H14), 7.87 (d, J = 16.3 Hz, 1H, H12), 7.82 (ddd, J = 8.3, 6.8, 1.1 Hz, 1H, H2), 7.67 (ddd, J = 8.2, 6.9, 1.1 Hz, 1H, H1), 7.61 (m, 1H, H18), 7.59 (d, J = 16.3 Hz, 1H, H11), 7.49 (dt, J = 10.3, 8.6 Hz, 1H, H15). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.0, 155.4, 150.8, 150.8, 149.5, 149.5, 149.3, 148.9, 148.9, 137.3, 134.5, 133.0, 130.6, 129.8, 128.1, 125.9, 125.2, 124.0, 121.2, 118.4, 118.3, 116.2, 116.1. MS: 311.1 (M^+). Calcd. Analysis for $C_{18}H_{11}F_2NO_2$: C, 69.45; H, 3.56; N, 4.50. Found: C, 69.39; H, 3.65; N, 4.43.

4.1.9 | (*E*)-2-(2,3-*di*fluorostyryl) quinoline-4-carboxylic acid 10

Yield 72%, yellow powder, mp 289.7°C-290.2°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 14.00 (s, 1H, H23), 8.64 (dd, J = 8.5;0.7 Hz, 1H, H3), 8.25 (s, 1H,H9), 8.12 (dd, J = 8.4; 0.5 Hz, 1H, H6), 7.98 (d, J = 16.3 Hz, 1H,H12), 7.84 (dt, J = 7.0;1.5 Hz, 1H, H2), 7.77 (dt, J = 7.0;1.5 Hz, 1H, H1), 7.72 (d, J = 16.3 Hz, 1H, H11), 7.69 (dt, J = 8.3;1.2 Hz, 1H, H14), 7.45 (dc, J = 8.2;1.2 Hz, 1H, H15), 7.45 (dc, J = 8.0;1.1 Hz, 1H, H16). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.9, 167.9, 160.3, 155.0, 151.5, 149.7, 149.3, 149.2, 148.9, 148.5, 147.7, 137.7, 136.4, 132.3, 132.3, 130.7, 130.1, 130.0, 129., 128.3, 127.6, 126.6, 126.5, 125.9, 125.8, 125.7, 125.6, 125.5, 124.8, 124.2, 123.9, 123.4, 123.1, 121.7, 117.8, 117.7. IR (cm^{-1}): ν 3420, 3060, 1656, 1485, 1250, 976, 780. Calcd. Analysis for $C_{18}H_{11}F_2NO_2$: C, 69.45; H, 3.56; N, 4.50. Found: C, 69.37; H, 3.62; N, 4.41.

4.1.10 | (*E*)-2-(2,6-*di*fluorostyryl) quinoline-4-carboxylic acid 11

Yield 60%, yellow powder, mp 284.6°C-285.0°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.63 (d, J = 8.5 Hz, 1H, H3), 8.22 (s, 1H, H9), 8.13 (d, J = 8.4 Hz, 1H, H6), 7.90 (d, J = 16.5 Hz, 1H, H12), 7.84 (t, J = 7.6 Hz, 1H, H2), 7.71 (t, J = 8.3, 1H, H1), 7.68 (d, J = 16.5 Hz, 1H, H11), 7.47 (m, 1H, H17), 7.24 (t, J = 8.6 Hz, 2H, H15,H16). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.9, 161.9, 160.2, 155.0, 148.8, 137.7, 134.3, 131.0, 130.7, 130.1, 128.3, 125.9, 124.3, 121.8, 120.9, 113.6, 112.8. IR (cm^{-1}): ν 3437, 3078, 1711, 1633, 1484, 1256, 1000, 984, 781. Calcd. Analysis for $C_{18}H_{11}F_2NO_2$: C, 69.45; H, 3.56; N, 4.50. Found: C, 69.39; H, 3.60; N, 4.44.

4.1.11 | (*E*)-2-(2,5-*di*fluorostyryl) quinoline-4-carboxylic acid 12

Yield 67%, yellow powder, mp 299°C-300°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 14.0 (s, 1H, H23), 8.65 (dd, J = 8.0, 0.6 Hz, 1H, H3), 8.21 (s, 1H, H9), 8.12 (d, J = 8.3 Hz, 1H, H6), 7.94 (d, J = 16.3 Hz, 1H, H12), 7.85 (td, J = 3.7 Hz, 1H, H14), 7.83 (td, J = 8.3, 7.0 Hz, H1), 7.74 (d, J = 16.3 Hz, 1H, H11), 7.69 (ddd, J = 8.2, 7.0,1.0 Hz, 1H, H2), 7.38 (td, J = 9.0, 4.5 Hz, 1H, H17), 7.27 (ddd, J = 9.0, 7.4, 3.5 Hz, 1H, H16). ^{13}C NMR (151 MHz, DMSO-d_6) δ (151 MHz, DMSO) δ 167.9, 159.7, 158.2, 157.7, 156.1, 155.0, 148.9, 137.6, 132.2, 132.1, 130.7, 130.0, 128.3, 125.9, 125.7, 124.3, 121.7, 117.4, 114.5. IR (cm^{-1}): ν 3420, 3060, 1656, 1485, 1250, 969, 780. Calcd. Analysis for $C_{18}H_{11}F_2NO_2$: C, 69.45; H, 3.56; N, 4.50. Found: C, 69.40; H, 3.59; N, 4.48.

4.1.12 | (*E*)-2-(3-fluorostyryl)quinoline-4-carboxylic acid 13

Yield 89%, yellow powder, mp 299.3°C-299.9°C (lit. 265°C-266°C).[27] ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.64 (d, J = 8.4 Hz, 1H, H3), 8.25 (s, 1H, H9), 8.09 (d, J = 8.4 Hz, 1H, H6), 7.91 (d, J = 16.3 Hz, 1H, H12), 7.83 (dt, J = 8.4, 1.2 Hz, 1H,), 7.70 to 7.59 (m, 3H), 7.64 (d, J = 16.3 Hz, 1H, H11), 7.48 (c, J = 7.8 Hz, 1H, H15), 7.20 (dt, J = 8.5, 2.4 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.0, 163.8, 162.2, 155.5, 148.9, 139.2, 139.2, 137.4, 133.9, 133.9, 131.2, 130.6, 129.9, 128.1, 125.9, 124.3, 121.3, 115.9, 113.9. Chemical Formula: $C_{18}H_{12}FNO_2$.

4.1.13 | (*E*)-2-(4-bromo-3,5-*di*methoxystyryl)quinoline-4-carboxylic acid 18

Yield 60%, orange powder, mp 279°C-280°C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.68 (d, J = 8.4 Hz, 1H, H3), 7.95 (m, 2H, H9;H6), 7.81 (d, J = 16.2 Hz, 1H, H12), 7.70 (d, J = 7.2 Hz, 1H, H2), 7.66 (d, J = 16.2 Hz, 1H, H11), 7.50 (t, J = 7.4, Hz, 1H, H1), 7.18 (s, 2H, H14;H18), 3.94 (s, 6H, H24;H26). ^{13}C NMR (151 MHz, DMSO-d_6) δ 169.3, 157.2, 155.5, 148.9, 137.7, 133.6, 130.6, 129.5, 129.1, 127.8, 126.1, 125.2, 118.7, 104.5, 100.4, 57.0 (C24;C26). IR (cm^{-1}): ν 3422, 3062, 2969, 1641, 1609, 1516, 1328, 1297, 984, 781. Calcd. Analysis for $C_{20}H_{16}BrNO_4$: C, 57.99; H, 3.89; N, 3.38. Found: C, 57.94; H, 3.92; N, 3.34.

4.2 | Biological assays

4.2.1 | *L. (L.) amazonensis* culture

L. (L.) amazonensis (IFLA/BR/67/PH8) were propagated as promastigotes at 26°C in M199 media supplemented with 5% penicillin/streptomycin, 0.1% hemin (25 mg mL^{-1} in 0.1 N NaOH), 10 mM adenine, and 10% FBS, pH 7.4. In the experiments, 7 days cultures (stationary phase) were used. Parasites were washed three times in PBS before use in experiments.

4.3 | Compounds activity against promastigotes assessed by MTT assay

The compounds' activity was determined by incubating *L. (L.) amazonensis* promastigotes in the presence of different compounds (29.3 μM—EC_{50}, previously determined). The number of viable cells was determined as described. Briefly, after 24, 48, and 72 hours of treatment, promastigotes were incubated with MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide; Sigma-Aldrich) and the optical density was determined in a plate reader (POLARstar Omega, BMG Labtech) with a reference wavelength of 690 nm and a test wavelength of 595 nm. Results were expressed as the mean percentage viability of treated parasites compared with untreated parasites (Control and DMSO control). Positive control treatments were performed by incubating the parasite with 2 μM of amphotericin B (Sigma-Aldrich) or 30 μM miltefosine (Sigma-Aldrich).

4.4 | Intracellular multiplication of parasites in treated bone marrow macrophages

BMMs were prepared as previously described. Briefly, BMMs from female mice were obtained after 7 days of differentiation of bone marrow in RPMI media (Gibco) supplemented with 20% (v/v) FBS and 20% (v/v) of L-929 cell supernatant. For macrophage infection, 8×10^4 (cytotoxic assay) or 2×10^5 (fluorescence assay) BMMs were plated in 96 well or 24 well dishes, respectively, 24 hours prior to experiments.

For the cytotoxic assay, BMMs were incubated with the compounds for 24, 48, and 72 hours, and the viability was accessed using MTT assay as described before.

For fluorescence assay, BMMs were plated on glass coverslips in dishes. Promastigotes were added at an MOI = 5 in RPMI supplemented with 10% (v/v) FBS and 2% (v/v) of L-929 cell supernatant for 2 hours at 34°C. BMMs were then washed three times with PBS and incubated for 24 hours. Later, the BMMs were incubated for 48 hours at 34°C in the presence of the compounds **1**, **2**, **3**, **4**, **5**, **6**, and **14**. Coverslips were then fixed with methanol 100%. The number of intracellular parasites was quantified by counting the total number of macrophages, infected macrophages, and the total number of intracellular parasites. The results were expressed as a number of parasites per number of 100 infected BMMs or the percentage of infected cells. Control treatment was performed by incubating the different forms of the parasite with Amphotericin B.

For fluorescence assay, Methanol-fixed cells were blocked with Saponine/BSA/TBS (0.1%) and incubated with 10 μg mL^{-1} of Propidium Iodide (Sigma-Aldrich) for 1 hour. Images were acquired by fluorescence microscopy DMI6000B/AF6000 (Leica) coupled to a digital camera system (DFC 365 FX).

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DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

ORCID

Silvia E. Asís https://orcid.org/0000-0002-3978-8638

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SUPPORTING INFORMATION

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