Synthesis and antileishmanial activity of styrylquinoline-type compounds: *in vitro* and *in vivo* studies

F.A. Petro-Buelvas, C.A. Guzmán*, H.S. Villa, S.M. Robledo, I.D. Vélez and G.G. Santafé

**Highlights**

- Four synthetic styrylquinolines were evaluated against leishmaniasis *in vitro* and *in vivo*.
- Of the four compounds evaluated *in vitro*, two were active against intracellular amastigotes.
- Two compounds show good *in vivo* activity against *L. (V) panamensis*.
- The compounds are promising to further improve their activity against the *Leishmania* parasite.
Synthesis and antileishmanial activity of styrylquinoline-type compounds: *in vitro* and *in vivo* studies

F.A. Petro-Buelvas1, C.A. Guzmán1,2, H.S. Villa3, S.M. Robledo2, I.D. Vélez2 and G.G. Santafé3

1IDEFARMA, Department of Regency and Pharmacy, University of Cordoba, Montería, Colombia.
2PECET Medical Research, Faculty of Medicine, University of Antioquia, Medellín Colombia.
3PRONAT, Department of Chemistry, University of Cordoba, Montería, Colombia.

Received: 30/05/2020 ; Accepted: 01/05/2021

Abstract: In this research, four styrylquinolines were synthesized and evaluated *in vitro* and *in vivo* antileishmanial activity. The following compounds were obtained: 2-[(E)-2-(2-chlorophenyl)ethenyl]quinoline-8-ol (3a), 4-bromo-2-[(E)-2-(8-hydroxyquinoline-2-yl)ethenyl]phenylacetate (3b), 4-[(E)-2-(8-hydroxyquinoline-2-yl)ethenyl]-2,6-dimethoxyphenylacetate (3c), 2-[(E)-2-[(2-acetyloxy)-5-nitrophenyl]ethenyl]quinoline-8-yl-acetate (3d). Of the four compounds, evaluated *in vitro*, 3b and 3c were active against intracellular amastigotes at median effective concentrations (EC50) of 2.8 and 3.14 μg mL−1 and exhibited toxicity over human U-937 macrophages at median lethal concentrations (LC50) of 5.5 and 3.4 μg mL−1, which showed selectivity indices (SI) of 1.96 and 1.08. The results of *in vivo* studies of therapeutic response in hamsters with experimental CL caused by *L. (V) panamensis* indicated that with topical creams 3b and 3c produced a clinical improvement of 4/5 (80%) of hamsters with 3b, and with 3c a complete cure of 1/5 (20%) and a clinical improvement of 2/5 (40%) of the hamsters, while the *in vivo* control drug (MA-il) produced clinical improvement in 3/5 (60%) of hamsters.

Keywords: Antileishmanial; styrylquinoline; cytotoxicity; *Leishmania*, quinoline.

INTRODUCTION

The term leishmaniasis describes a set of diseases caused by protozoans from the *Leishmania* genus. These are manifested in three different clinical forms: cutaneous, mucocutaneous, and visceral in function of the carrier’s immune response and of the parasite species. The leishmaniasis is a zoonosis transmitted by a female phlebotomine vector in which mammals are the most frequent host reservoirs. Visceral leishmaniasis is the most severe clinical form of the disease with mortality close to 100% if untreated; the cutaneous and mucocutaneous forms are not as severe, but can lead to disfiguring lesions (WHO,2019). Currently, treatments are carried out based on medications, like Amphotericin B (AmB), Sitamaquine, Meglumine antimoniate (MA), Miltefosine, Parmomycin, and Pentamidine; as fundamental part of the structure of these active principals, we can highlight the presence of quinolinic compounds, which in addition to demonstrating their activity against leishmaniasis (Richard and Werbovetz, 2010) have also exhibited other types of activities, such as anti-trypanosomal, anti-tumor, bactericide, anti-mycobacterial, fungicide, anti-asthmatic, anti-plasmodial and anti-VIH (Franck et al., 2004; García et al., 2010; Musioli et al., 2010; Delattin et al., 2012; Marella et al., 2013; Upadhayaya et al., 2013; Afzal et al., 2015; Kieffer et al., 2015). Today, an important number of researches on antileishmanial substances have to do with isolating – in the case of natural products – or obtaining – in the case of organic synthesis – of molecules with quinolinic or isoquinolinic nuclei that can serve as therapeutic alternatives in treating this disease. Among the most promising compounds, *in vitro* and *in vivo* assays, the following are highlighted:

- 8-aminoquinoline (Sitamaquine), styrylquinoline-type compounds and derivatives reduced from them, 2-arylquinolines, quinoline dimers and quinolinetriclosan hybrids, and triazine indol-quinolines, all with antileishmanial activity (Mesa et al., 2008; Pinheiro et al., 2010; Arango et al., 2011; Sánchez et al., 2014; Sharma et al., 2014; Singh et al., 2014).

The previous aspects suggest the importance and applicability of quinolinic nuclei; therefore, this research synthesized styrylquinoline-type molecules determining their antileishmanial activity and their cytotoxicity through *in vitro* and *in vivo* trials to test their effectiveness in countering the pathological effects and, thus, contribute to development of new treatments and relieving the disease burden, which poses a global public health problem. In this context, the styrylquinolines were obtained using the Perkin-type condensation reaction and their structure was corroborated using the nuclear magnetic resonance spectroscopic technique in one and two dimensions. These compounds were evaluated for antileishmanial activity *in vitro* against intracellular amastigotes and *in vivo* in murines infected with the parasite and, in turn, their toxicity was monitored by measuring serum values of ALT (alanine amino-transferase), BUN (Blood urea nitrogen) and creatinine, showing that two of the synthesized compounds showed promising antileishmanial activity, sufficient to be considered LEAD compounds. Based on its potential,
you should continue to optimize your formulation and therapeutic dose.

MATERIALS AND METHODS

General procedure to synthesize styrylquinolines

The styrylquinolines were obtained by means of a Perkin-type condensation reaction using high purity reagents from 8-hydroxyquinoline (1) with 6.00 mL of acetic anhydride (Ac₂O) adding the corresponding aromatic aldehyde: 2-chlorobenzaldehyde (2a), 5-bromo-2-hydroxybenzaldehyde (2b), 4-hydroxy-3,5-dimethoxybenzaldehyde (2c) or 2-hydroxy-5-nitrobenzaldehyde (2d) in stoichiometric excess with respect to 1 (Figure 2) and heated under reflux with reflux with constant stirring and the progress of the reaction was monitored by thin layer chromatography (TLC) to verify the consumption of precursor and the formation of the product and the time used for the syntheses was between 8 and 14 h. Upon completing the reaction, the mixture was left to cool at room temperature and sufficient amount of a saturated solution of NaHCO₃ was added until hydrolyzing the remnant Ac₂O. Extraction was conducted with petroleum benzine (PB) and ethyl acetate (EtOAc) in 1:2 ratio; the organic phase was dried with anhydrous Na₂SO₄, filtered, and concentrated at reduced pressure. Finally, the raw product was purified by column chromatography using PB: EtOAc as the eluent with increasing polarity gradient raw product was purified by column chromatography using PB: EtOAc as the eluent with increasing polarity gradient.

Structural characterization

Structural characterization was conducted through nuclear magnetic resonance (NMR) spectroscopy in Bruker Advance DRX (300 MHz) equipment operating at 300 MHz for ¹H and at 75 MHz for ¹³C, using deuterated chloroform (CDCl₃) as the solvent. Chemical shifts (δ) are expressed in ppm, having tetramethylsilane (TMS) as the internal standard and the coupling constants (J) in Hertz (Hz).

Figure 1: Styrylquinoline.
Biological activity

Cells and culture conditions

The U-937 promonocytes (CRL-1593.2™) (American Type Culture Collection) were cultured in complete medium containing RPMI -1640 (Sigma) supplemented with 10% fetal bovine serum (FBS) (Gibco, Life Technologies) and 1% antibiotics (100 U mL⁻¹ of penicillin and 0.1 mg mL⁻¹ of streptomycin) (Sigma). The cells were under standard culture conditions at 37 °C, 5% CO₂ with medium change every 72 h until use.

Leishmania culture conditions

L. (V) panamensis (MHOM/CO/87/UA140-EGFP) was used for in vitro and in vivo assays (Pulido et al., 2012). Virulence was maintained through successive passages in golden hamsters (Mesocricetus auratus). Parasites were recovered from hamsters after aspirates of the skin lesions and culturing in biphasic Novy-MacNeal-Nicholle medium at 26 °C to obtain promastigotes. Parasites in stationary phase of growth (6-day in culture) were used to infect the U-937 cells and hamsters.

In vitro cytotoxicity

The U-937 cells in exponential growth phase were adjusted to a concentration of 1 x 10⁶ cells mL⁻¹ of complete RPMI-1640 medium. One hundred μL of cell suspension were dispensed in each well of a 96-well cell culture plate and then, 100 μL of complete RPMI-1640 medium with the corresponding concentrations of each styrilquinoline compound were added to each well. AmB, was included as cytotoxicity control. For all compounds and AmB were evaluated, six double serial dilutions from 200 to 3.125 μg mL⁻¹. Simultaneously, cells cultured in the absence of the compounds and kept under the same conditions were used as viability control (negative control) (Robledo et al., 1999; Weniger et al., 2001). After 72 h of incubation, cell mortality was determined by using the MTT method and reading the optical densities in a spectrophotometer at 570 nm (Serenò and Lemesre, 1997). The optical densities obtained were used to calculate the percentage of mortality. Tests were run at least twice with three replicates for each concentration evaluated.

In vitro antileishmanial activity on amastigotes of L. (V) panamensis

The U-937 cells at a concentration of 300,000 cells mL⁻¹ kept in complete RPMI 1640 medium with 0.1 μg mL⁻¹ of phorbol myristate acetate were seeded on 24-well cell culture plates and incubated at 37 °C and 5% CO₂. Cells were infected with promastigotes of L. (V) panamensis-EGFP in stationary growth phase (MOI: 20 parasites per cell), and the plates were incubated for 3 h at 34 °C and 5% CO₂. The medium was removed, the cells were washed with PBS, and fresh complete medium was added. The plates were incubated for another 24 h at 34 °C and 5% CO₂. The medium was replaced by fresh medium containing each compound at 100, 50, 25, 12.5, 6.25, 3.125 or 1.56 μg mL⁻¹ and the plates were incubated at 34 °C and 5% CO₂. After 72 h of incubation, the effect of the compounds was determined on the viability of the intracellular amastigotes through flow cytometry with readings at 488 nm of excitation and 525 nm emission with an Argon laser capturing the percentage of positive events for green fluorescence (Sereno and Lemesre, 1997; Varela et al., 2009). AmB was used as a positive assay control and infected but non exposed cells were used as the control of infection and a negative assay control. The tests were conducted at least twice with a minimum of three replicates per concentration evaluated. The number of positive GFP events and the median fluorescence intensity (MFI) corresponded to the amount of live parasites (parasitic load). This number was used to calculate the percentage of inhibition for each concentration evaluated with respect to the untreated control (Serenò and Lemesre, 1997; Robledo et al., 1999).

Topic formulations of 3b and 3c

Creams were prepared based on a mixture of glycerin, vaseline, emulgade® 1000, Cetyl alcohol and surfactant and then, the pure compounds 3b or 3c were subsequently incorporated into the base cream at a concentration of 2%.

In vivo therapeutic response of a cream formulation containing 2% 3b and 2% 3c

Male and female, 6-weeks old hamsters were anesthetized (ketamine 40 mg/kg and xylazine 5 mg/kg) and then inoculated in the dorsal skin with 5 × 10⁵ promastigotes of L. (V) panamensis-EGFP in 100 μL PBS as described elsewhere (Robledo et al., 2012). After a cutaneous ulcer was developed hamsters were distributed in three experimental groups (n = 5 each): two groups of hamsters were treated via topic with 40 mg of 2% 3b or 2% 3c cream, administered every day for 28 days. The third group of hamsters was treated with 200 mg of MA intralesional, administered two times a week for 4 weeks (28 days). The time points of evaluation were: before treatment (treatment day 0), end of treatment (treatment day 28), and post treatment days (PTD) 30, 60, and 90. Animal welfare was supervised daily during the study. The effectiveness of each treatment was determined by comparing the lesion sizes after treatment respect to before treatment. Clinical outcomes were recorded at the end of study as cure (healing of 100% of the area and complete disappearance of the lesion); improvement (reducing the size of the lesion in >30% of the area) or failure (increasing the size of the lesion). Toxicity of cream preparations was determined according to changes in the body weight during the study and levels of alanine amino-transferase (ALT), blood urea nitrogen (BUN), and creatinine in serum at TDO and PTD8, measured using commercial kits (LabCAM) (Robledo et al., 2012).

Statistical analysis

Cytotoxicity was determined according to the percentages of viability and mortality registered for each compound, including AmB and culture medium. Percentage of viability was calculated based on the ratio between O.D of exposed cells vs. control cells using the equation:
% Mortality = 100 - [(O.D exposed cells / O.D non-exposed cells) × 100].

The mortality % were used to calculate the LC\textsubscript{50} that corresponds to the concentration necessary to eliminate 50% of cells using Prism 6.0 (Graph pad Prism, San Diego, CA, USA).

On the other hand, the antileishmanial activity was determined according to the percentage of infected cells that correspond to the positive events in a dot plot analysis with the green fluorescence (parasites) in y-axis and the Forward Scatter (FSC) in x-axis. Then, the amount of parasites in infected cells was determined according to the MFI of those fluorescent parasites. Lastly, the parasite inhibition percentage was calculated by the ratio between the MFI of exposed parasites vs. non exposed infected cells using the equation:

% inhibition of infection = 100 - [(MFI exposed infected cells / MFI non-exposed infected cells) × 100].

Results of antileishmanial activity were expressed as EC\textsubscript{50} determined using Prism 6.0 (Graph pad Prism, San Diego, CA, USA). In turn, the selectivity index (SI), was calculated by the ratio obtained between the activity observed in cells (LC\textsubscript{50}) and the activity obtained in parasites (EC\textsubscript{50}), using the formula: SI = LC\textsubscript{50}/EC\textsubscript{50}.

Values are expressed as mean ± SD. In order to compare the different compounds in terms of their biological activity, both cytotoxicity and antileishmanial activity were classified as high, moderate or low, according to the LC\textsubscript{50} and EC\textsubscript{50} values, respectively. In this way, the cytotoxicity was considered high when the LC\textsubscript{50} was < 100 μg; moderate when the LC\textsubscript{50} was between 100 and 200 μg and low when the LC\textsubscript{50} was > 200 μg. On the other hand, the antileishmanial activity was considered high when the EC\textsubscript{50} was < 25 μg, moderate when the EC\textsubscript{50} was between 25 and 50 μg and low when the EC\textsubscript{50} was > 50 μg (Murillo et al., 2019). Data from in vivo study were analyzed by a two-way ANOVA using Prism 6.0 (Graph pad Prism, San Diego, CA, USA). Differences were considered significant if p < 0.05 (Robledo et al., 2012).

**Ethical issues**

The procedures involving laboratory materials were manipulated and discarded following the Institutional Biosafety Manual. The procedures involving animals were approved by the Ethical Committee for the experimentation in animals of the University of Antioquia (Act No. 91 of September 25, 2014).

**RESULTS**

**Chemistry**

Four compounds were synthesized (3a-d): 2-[(\textit{E})-2-(2-chlorophenyl)ethenyl]quinolin-8-ol (3a), 4-bromo-2-[(\textit{E})-2-(8-hydroxyquinolin-2-yl)ethenyl]phenylacetate (3b), 4-[(\textit{E})-2-(8-hydroxyquinolin-2-y1)ethenyl]-2,6-dimethoxyphenylacetate (3c), 2-[(\textit{E})-2-(2-acetyloxy)-5-nitrophenyl]ethenyl]quinolin-8-yl-acetate (3d) with yields above 60% (Figure 2).

![Figure 2: Synthesis of styrylquinolines (3a-d).](image-url)
Table 1: Antileishmanial activity against intracellular amastigotes and cytotoxicity of the synthesized compounds

| Compound | Cytotoxicity LC_{50} (µg mL^{-1}) | Antileishmanial activity EC_{50} (µg mL^{-1}) | ^{a}SI |
|----------|------------------------------------|-----------------------------------------------|--------|
| 3a       | 1.70 ± 0.20                        | >2                                            | <0.85  |
| 3b       | 5.50 ± 0.20                        | 2.80 ± 0.40                                   | 1.96   |
| 3c       | 3.40 ± 0.40                        | 3.14 ± 0.40                                   | 1.08   |
| 3d       | 10.50 ± 1.90                       | 36.49 ± 6.35                                  | 0.29   |
| AmB      | 52.50 ± 3.60                       | 0.05 ± 0.01                                   | 1044   |

Data correspond to the average values ± standard deviation of LC_{50} and EC_{50}. ^{a}IS: Selectivity index = LC_{50}/EC_{50}.

In vitro cytotoxicity and Antileishmanial activity of Styrylquinolines

Cytotoxicity was evaluated against human U-937 cells with for each compound the results were expressed as the median lethal concentration (LC_{50}) (Table 1). According the defined classification, all compounds were cytotoxic with LC_{50} values lower than 11 µg mL^{-1} (Table 1). Compound 3a had the highest toxicity (LC_{50} = 1.7 µg mL^{-1}) and compound 3d had the lowest cytotoxicity (LC_{50} = 10.5 µg mL^{-1}).

In vitro antileishmanial activity of the compounds was evaluated against amastigotes of L. (V) panamensis obtained after infection of U-937 cells (Table 1). Only compounds 3b and 3c showed very high activity against L. (V) panamensis with EC_{50} values of 2.80 and 3.14 µg mL^{-1} respectively. As expected, AmB was active also at an EC_{50} of 0.05 µg mL^{-1}. All compounds exhibited a lower selectivity index than the control drug (AmB), with the best being 3b and 3c with values 1.96 and 1.08 respectively.

In vivo therapeutic response and toxicity of 3b and 3c topic formulation

Ninety days after the end of treatment (PTD90), corresponding to the end of the study, the treatment of hamsters with the 2% cream formulation of 3c during 28 days allowed healing (cure complete and re-epithelialization of damaged skin) of 1/5 of the hamsters and clinical improvement of 2/5 of the hamsters; the remainder hamsters failed to this treatment (Figure 3b). On the other hand, treatment with 2% 3b cream allowed clinical improvement of 4/5 hamsters with reduction in the size of the lesions varying between 13.2% and 34.1%, while remaining two hamsters failed to show improvements with this treatment (Figure 3a). In the group of hamsters treated with intralesional meglumine antimoniate (MA-il) 3/5 (60%) hamsters showed a reduction in lesion size from 19.1% to 64.2% and two hamsters failed on this treatment (Figure 3c).

Figure 3: Lesion size in golden hamsters infected with L. panamensis-EGFP promastigotes. (a-c) show the evolution of lesions in dorsum of the hamsters that received 3b, 3c and MA-il respectively.
Figure 3 shows the increase or decrease in the sizes of lesion during the study. $y$ axis corresponds to area of lesion in mm$^2$ and $x$ axis corresponds to days of follow up. *TD0 (Treatment- day 0); TD28 (Treatment- day 28, the last day of treatment); PTD30 (Post-treatment -day 30); PTD60 (Post-treatment -day 60); PTD90 (Post-treatment -day 90).

Treatment with 2% cream of 3b in 5 murines of different sex (Figure 3a), treatment with 2% cream of 3c in 5 murines of different sex (Figure 3b) and the treatment with MA-il in 5 murines of different sex (Figure 3c).

A decrease of the size after the treatment corresponds to a positive therapeutic response, while an increase is indicative of negative response to the treatment. A positive therapeutic response is associated to clinical improvement and cure while a negative response is associated to a failure.

The appearance of the lesions of one hamster with cure with 3c and another with clinical improvement after the treatment with 3b compound are illustrated in Figure 4a and 4b respectively.

In general, hamsters treated with 3b, 3c or MA-il gained weight during the study ranging from 0.6% to 16.5% in hamsters treated with 3b treatment, from 5.6% to 21.7% in hamsters treated with 3c and from 4.6% to 18.8 in hamsters treated with MA-il (Figure 5). This difference was statistically significant ($p < 0.05$).

Figure 5 shows the evolution of the body weight of hamsters during the study measured before the treatment, at the end of the treatment and every 15 days after completing the treatment. $y$ axis corresponds to the weight in grams and $x$ axis corresponds to days of follow up. * $p < 0.05$ MA-il vs other groups. TD0 (Treatment- day 0), TD28 (Treatment- day 28, end of treatment); PTD15 (Post-treatment -day 15); PTD30 (Post-treatment -day 30); PTD45 (Post-treatment -day 45); PTD60 (Post-treatment -day 60); PTD75 (Post-treatment -day 75) and PTD90 (Post-treatment -day 90).

Serum levels of ALT, BUN and creatinine were within the range of normal values for all animals both before (TD0) and after treatment (PTD8) (Figure 6).

The results are presented as the average ± standard error. The following biomarkers were measured in hamster serum: a alanine amino-transferase (ALT), b blood urea nitrogen (BUN), and c creatinine. $p > 0.05$ One-way ANOVA with Bonferroni’s correction. The normal range of measurements is indicated by a gray zone.

![Figure 4: Outcome of lesion in golden hamsters infected with *L. panamensis*-EGFP promastigotes. a) the evolution of lesions in dorsum of the hamsters that received 3c and b) the evolution of lesions in dorsum of the hamsters that received 3b before treatment (TD0), at the end of treatment (TD28) and, at the end of the study (PTD90).](image)

![Figure 5: Toxicity assessment of 3b, 3c and MA-il in golden hamsters by measuring the body weight. Figure shows the evolution of the body weight (g); all data have been reported as the mean ± SD, 5 hamsters per group.](image)
The alanine amino-transferase (ALT) levels measured at the beginning of the treatment (TD0) and after 8 days (TD8) showed slight increases with the treatment with 3c and the control drug (MA-il), however they did not exceed the critical levels (gray area) (Figure 6a). The measurement of blood urea nitrogen (BUN) at the beginning of the treatment (TD0) and after 8 days (TD8) did not show important variations with the treatment with 3b and 3c and neither with the control medicine (MA-il), thus presenting acceptable levels within the allowed (gray area) (Figure 6b). The creatinine measured in the hamsters did not show significant variations with the treatment with 3c and with the control drug (MA-il), however it was slightly increased with the treatment with 3b, but this did not represent levels outside the normal values (gray area) (Figure 6c) (Robledo et al., 2012).

**DISCUSSION**

Four styrylquinolines were obtained of which two showed promising activity against *L. (V) panamensis*, 4-bromo-2-[(E)-2-(8-hydroxyquinoline-2-yl)ethenyl]phenyl acetate (3b) and 4-[(E)-2-(8-hydroxyquinoline-2-yl)ethenyl]-2,6-dimethoxyphenyl-acetate (3c) with EC$_{50}$ of 2.8 and 3.14 µg mL$^{-1}$, respectively, compared to AmB, with EC$_{50}$ of 0.05 µg mL$^{-1}$.

Similar studies by Sánchez et al., 2014 show that styrylquinoline-type compounds, like: 2-[(E)-2-(2,3-diacetoxyloxy)ethenyl]quinoline-8-ylacetate and 2-[(E)-2-(4-acetoxy-3-methoxyphenyl)ethenyl]quinoline show good in vitro activity against *L. (V) panamensis* with SI below 1.0 for both compounds. Upon comparing the results from said research to those from this work, better in vitro antileishmanial activity is evident, with SI above 1.0 (1.96 and 1.08). It was also established that synthetic compounds 3b and 3c had better activity than styrylquinoline 2-[(E)-2-phenylethenyl]quinoline-8-ol and (4-[(E)-2-[8-(acetoxy)quinoline-2-yl]ethenyl]-1,2-phenylene) diacetate, obtained by Loiseau et al., 2011, with SI of 0.6 and 0.7, respectively, against *Leishmania donovani*, which is a different species from *L. (V) panamensis* that belongs to the *Leishmania* subgenus and is the causal agent of visceral leishmaniasis (Loiseau et al., 2011).

Compound 3a due to its high in vitro cytotoxicity and a selectivity index below unity, in vivo antileishmanial activity was not evaluated. Compound 3d showed the lowest antileishmanial activity of the evaluated compounds, for this reason it was not evaluated in the in vivo phase, however, due to its low toxicity, it could be considered a good molecular basis to be synthetically modified and improve its effectiveness against the parasite and similarly, for 3a to reduce its cytotoxicity.

Although compounds 3b and 3c showed a lower value of EC$_{50}$ than AmB, both compounds are still highly active at levels below 4 µg mL$^{-1}$. On the other hand, AmB was used as an internal control of cytotoxicity and leishmanicidal activity to guarantee the correct functioning of the in vitro assay. Rather than find molecules with lower levels of activity than AmB, the purpose of projects involved in the search of novel therapeutic alternatives is find molecules that have activity in the accepted ranges (p.e < 20 µg mL$^{-1}$, according our scale). Moreover, it has been shown that all different *Leishmania* strains and species have different sensitivity to each one of the antileishmanial drugs (Palacio et al., 2017). Similarly, toxicity of compounds 3b and 3c was higher than that showed by
AmB; nevertheless, when used in a cream formulation neither compound showed signs associated with toxicity in the treated hamsters.

The antileishmanial potential of these compounds was validated through in vivo studies, evidencing that the majority of the hamsters treated with 3b or 3c responded positively to treatment and showed important clinical improvement. Further studies are needed to optimize aspects related to the formulation, doses and duration of treatment. Although different quinoline derivative compounds have been tested for antileishmanial activity, the in vivo activity for these compounds are reported for first time.

CONCLUSIONS

Compounds 3b and 3c showed good antileishmanial activity in vitro and although they did not exceed the control drug (AmB), they presented good selectivity indices, which allowed preliminary studies in hamsters with CL caused by L. (V) panamensis and topical creams at 2% of each synthetic compound were used, they produced a clinical improvement of 4/5 (80%) of hamsters with 3b and 3c, a complete cure of 1/5 (20%) and a clinical improvement of 2/5 (40%) of the hamsters, while the in vivo control drug (MA-il) produced a clinical improvement in 3/5 (60%) of the hamsters with wound reduction percentages similar to 3b and 3c, which which is a very important result.

These results suggest that compounds 3b and 3c could be considered LEAD compounds; with the potential to continue optimizing the formulation, dosage and therapeutic regimen processes that allow their use as new strategies in the treatment and cure of CL.

ACKNOWLEDGMENTS

This research was funded by the University of Cordoba and the University of Antioquia. Thanking Professor Omar Torres Ayazo (R.I.P) for his leadership in this research topic.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Afzal, O., Kumar, S., Haider, M., Ali, M., Kumar, R., Jaggi, M. and Bawa A. (2015). Review on anticaner potential of bioactive heterocycle quinoline. European Journal of Medicinal Chemistry 97: 871-910. DOI: 10.1016/j.ejmech.2014.07.044.

Arango, V., Domínguez, J., Cardona, W., Robledo, S., Muñoz, D., Figadere, B. and Sáez, J. (2011). Synthesis and leishmanicidal activity of quinoline–triclosan and quinoline–eugenol Hybrids. Medicinal Chemistry Research 21: 3445-3454. DOI: 10.1007/s00044-011-9886-8.

Delattin, N., Bardiot, D., Marchand, A., Chaltin, P., De Brucker, K., Cammue, B. and Thevissen, K. (2012). Identification of Fungicidal 2,6-disubstituted quinolines with activity against candida biofilms. Molecules 17: 12243-12251. DOI: 10.3390/molecules171012243.

Finney, J.D. (1978). Statistical method in biological assay, 3rd ed. Charles Griffin & Co press, London, 508.

Franck, X., Fournet, A., Prina, E., Mahieux, R., Hoquetmiller, R. and Figadère, B. (2004). Biological evaluation of substituted quinolines. Bioorg. Bioorganic & Medicinal Chemistry Letters 14:3635-3638. DOI: 10.1016/j.bmcl.2004.05.026.

Garcia, P., Genes, C., Molano, P., Torres, O., Sáez, J. and Triana O. (2010). Evaluation of the trypanocidal, cytotoxic and genotoxic activity of styrylquinoline analogs. Journal of Chemotherapy 22: 169-174. DOI: 10.1179/joc.2010.22.3.169.

Kiefer, C., Cohen, A., Verhaeghe, P., Hutter, M.S., Castera-Ducros, C., Laget, M., Remusat, V., M’Rabet, M., Rault, S. and Rathelot, P. (2015). Looking for new antileishmanial derivatives in 8-nitroquinolinol-2(1H)-one series. European Journal of Medicinal Chemistry 92: 282-294. DOI: 10.1016/j.ejmech.2014.12.056.

Loiseau, P., Gupta, S., Verma, A., Srivastava, S., Puri, S., Sliman, F., Normand-Bayle, M. and Desmalle, D. (2011). In vitro activities of new 2-substituted quinolines against Leishmania donovani. Antimicrobial agents and chemotherapy 55: 1777-1780. DOI: 10.1128/AAC.01299-10.

Marella, A., Tanwar, O., Saha, R., Ali, M., Srivastava, S., Akhter, M., Shaquequzzamman, M. and Ala, M. (2013). Quinoline: A versatile heterocyclic. Saudi Pharmaceutical Journal 21: 1-12. DOI: 10.1016/j.jsp.2012.03.002.

Mesa, A., Molano, P., Seon, B., Figadere, B., Robledo, S., Muñoz, D. and Sáez, J. (2008). Síntesis y actividades leishmanicida y citotóxica In Vitro de análogos 2-arilquinolinas. Vitae 15: 259-266.

Murillo, J.A., Gil, J.F., Upegui, Y.A., Restrepo, A.M., Robledo, S.M., Quiñones, W., Echeverri, F., San Martín, A., Olivo, H.F. and Escobar, G. (2019). Antileishmanial activity and cytotoxicity of ent-beyerene diterpenoids. Bioorganic & Medicinal Chemistry 27: 153-160. DOI: 10.1016/j.bmc.2018.11.030.

Musiol, R., Jampilek, J., Nycz, J., Pesko, M., Eichhorn, K., Kralova, K., Vejsova, M., O’Mahony, J., Coffey, A. and Mrozek, A. (2010). Investigating the activity spectrum for ring-substituted 8-hydroxyquinolines. Molecules 15: 288-304. DOI: 10.3390/molecules15010288.

Pinheiro, A., Souza, M. and Bispo, M. (2010). Importance of Quinoline Nucleus and Its Derivatives in Drug Discovery. Revista Fitos 5:53-63.

Palacios, G., Parodi, A., Upegui, Y.A., Montoya, A., Pulido, S., Vélez, I.D., Robledo, S.M. (2017). Studies in vitro on infectivity and sensitivity to antileishmanial drugs in New World Leishmania spp for the in vitro and in vivo screening of antileishmanial drugs. Acta Tropica 144:1718-1725. DOI: 10.1017/S0001182017000671.

Pulido, S.A., Muñoz, D.L., Restrepo, A.M., Alzate, J.F., Vélez, I.D. and Robledo, S.M. (2012). Improvement of the green fluorescent protein reporter system in Leishmania spp. for the in vitro and in vivo screening of antileishmanial drugs. Acta Tropica 122: 36-45. DOI: 10.1016/j.actatropica.2011.11.015.

Richard, J.K. and Werbovetz. (2010). New antileishmanial candidates and lead compounds. Current Opinion in Chemical Biology 14: 447-455. DOI: 10.1016/j.
F.A. Petro-Buelvas et al.

Robledo, S., Carrillo, L., Daza, A., Restrepo, A., Muñoz, D., Tobón, J., Murillo, J., López, A., Ríos, C. and Mesa, C. (2012). Cutaneous leishmaniasis in the dorsal skin of hamsters: A useful model for the screening of antileishmanial Drugs. *Journal of Visualized Experiments* **21**: 1-7. DOI: 10.3791/3533.

Robledo, S., Valencia, A., and Saravia, N. (1999) Sensitivity to glucantime of *Leishmania* viannia isolated from patients prior to treatment. *The Journal of parasitology* **85**: 360.

Sánchez, E., Santafé, G., Torres, O., Muñoz, D. and Robledo, S. (2014). Compuestos sintéticos tipo estirilquinolinas con actividades leishmanicida Y Citotóxica. *Biomédica* **34**: 605-611. DOI: 10.7705/biomedica.v34i4.2299.

Santafé, G., Sánchez, E. and Torres, O. (2016). Síntesis y actividad antimalárica de estirilquinolinas sobre plasmodium falciparum. *Información Tecnológica* **27**: 97-104. DOI: 10.4067/S0718-07642016000200012.

Sereno, D. and Lemesre, J. (1997). Use of an enzymatic micromethod to quantify amastigote stage of *Leishmania amazonensis* in vitro. *Parasitology Research* **83**: 401-403. DOI: 10.1007/s004360050272.

Sharma, R., Pandey, A., Shivahare, R., Srivastava, K., Gupta, S. and Chauhan, P. (2014). Triazino Indole–quinoline hybrid: A novel approach to antileishmanial agents. *Bioorganic & Medicinal Chemistry Letters* **24**: 298-301. DOI: 10.1016/j.bmcl.2013.11.018.

Singh, N., Mishra, B., Bajpai, S., Singh, R. and Tiwari, V. (2014). Natural product based leads to fight against leishmaniasis. *Bioorganic & medicinal chemistry* **22**: 18-45. DOI: 10.1016/j.bmc.2013.11.048.

Upadhyaya, R., Dixit, S., Földesi, A. and Chattopadhyaya, J. (2013). New antiprotozoal agents: Their synthesis and biological evaluations. *Bioorganic & Medicinal Chemistry Letters* **23**: 2750-2758. DOI: 10.1016/j.bmcl.2013.02.054.

Varela, M.R., Muñoz, D., Robledo, S., Kolli, B., Dutta, S., Chang, K. and Muskus, C. (2009). *Leishmania* (*Viannia*) panamensis: An in vitro assay using the expression of GFP for screening of antileishmanial drug. *Experimental Parasitology* **122**:134-139. DOI: 10.1016/j.exppara.2009.02.012.

Weniger, B., Robledo, S., Arango, G.J., Deharo, E., Aragón, R., Muñoz, V., Callapa, J., Lobstein, A. and Anton, R. (2001). Antiprotozoal activities of colombian plants. *Journal of Ethnopharmacology* **78**:193-200. DOI: 10.1016/S0378-8741(01)00346-4.

World Health Organization investing to overcome the global impact of neglected tropical diseases, Third WHO Report Neglected Diseases, 2015 WHO/HTM/NTD/2015-1https://www.who.int/neglected_diseases/9789241564861/en/; Accessed July 31, 2019.