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Synthesis and biological evaluation of new quinoline derivatives as antileishmanial and antitrypanosomal agents

Santiago N. Chanquia,^a Facundo Larregui,^a Vanesa Puente,^b Carlos Labriola,^c Elisa Lombardo,^{b*} and Guadalupe García Liñares^{a*}

^aLaboratorio de Biocatálisis. Departamento de Química Orgánica y UMYMFOR, Facultad de Ciencias Exactas y Naturales,Universidad de Buenos Aires, Ciudad Universitaria,Pabellón 2, piso 3, C1428EGA Buenos Aires, Argentina. ^b Centro de Investigaciones sobre Porfirias y Porfirinas (CIPYP, UBA-CONICET), Hospital de Clínicas José de San Martín, Avenida Córdoba 2351, 1120 Buenos Aires, Argentina.

^C Instituto de Investigaciones Bioquímicas, Av. Patricias Argentinas 435C1405BWE Buenos Aires, Argentina

Abstract

As a part of our project aimed at developing new safe chemotherapeutic agents against tropical diseases, a series of aryl derivatives of 2- and 3-aminoquinoline, some of them new compounds, was designed, synthesized, and evaluated as antiproliferative agents against Trypanosoma cruzi, the parasite responsible for American trypanosomiasis (Chagas' disease), and Leishmania mexicana, the etiological agent of Leishmaniasis. Some of them showed a remarkable activity as parasite growth inhibitors. Fluorine-containing derivatives **11b** and **11c** were more than twice more potent than geneticin against intracellular promastigote form of Leishmania mexicana exhibiting both IC₅₀ values of 41.9 μ M. The IC₅₀ values corresponding to fluorine and chlorine derivatives **11b-d** were in the same order than benznidazole against epimastigote form. These drugs are interesting examples of effective antiparasitic agents with outstanding potential not only as lead drugs but also to be used for further in vivo studies. In addition, the obtained compounds showed no toxicity in Vero cells, which makes them good candidates to control tropical diseases. Regarding the probable mode of action, assayed quinoline derivatives interacted with hemin, inhibiting its degradation and generating oxidative stress that is not counteracted by the antioxidant defense system of the parasite.

Keywords: quinoline derivatives; leishmaniasis; Chagas' disease; hemin interaction; oxidative damage

*Corresponding author. e-mail: linares@qo.fcen.uba.ar; elombardo@qb.fcen.uba.ar

1. Introduction

American trypanosomiasis (Chagas' disease) and leishmaniasis are among the most prevalent parasitic diseases worldwide [1]. It has been estimated that around 20 million people are infected and over 40 million individuals are at risk of infection by the hemoflagellated protozoan *Trypanosoma cruzi*, the responsible agent of Chagas' disease [2]. *T. cruzi* has a complex life cycle possessing four main morphological forms [3]. It multiplies within the insect gut as an epimastigote form and is disseminated as a non-dividing metacyclic trypomastigote. In the mammalian host, *T. cruzi* proliferates as the intracellular amastigote form, which is afterwards released into the bloodstream as a non-dividing highly infective trypomastigote [3, 4]. The mechanism of transmission in developed countries, where Chagas' disease is not endemic, is via the placenta, by blood transfusion or migration of individuals [5]. *T. cruzi* can also be orally trasnmitted by food ingestion [6].

Leishmaniasis is also considered as one of the most important parasitic diseases and, taking into account the number of infected, it is found second, after malaria [7, 8]. Leishmaniasis are a group of clinical diseases produced by at least 17 species and some mutants of the protozoan *Leishmania* and transmitted to the humans by the bite of the female phlebotomine sandfly [3, 9]. The disease comprises three major syndromes: cutaneous, mucocutaneous and visceral leishmaniasis. *Leishmania mexicana* is one of the species that produce cutaneous leishmaniasis [10]. It is estimated that there are almost 1.3 million new cases of leishmaniasis and around 30,000 deaths annually [8, 11, 12]. In addition, the incidence of leishmaniasis in urban areas [13, 14].

The existing chemotherapy either for Chagas' disease or leishmaniasis remains deficient. In the first case, the chemotherapy is based on two drugs empirically discovered, nifurtimox (1) and benznidazole (Bnz) (2) (Fig 1). Although both of these compounds are able to cure at least 50% of recent infections, they present severe side effects [15, 16].

The chemotherapy for leishmaniasis has been based for over 60 years on the use of pentavalent antimonial drugs being meglumine antimoniate (Glucantime) (3) or sodium stibogluconate (Pentostam) (4) the first-choice drugs (Fig 1) [17]. However, these drugs are not satisfactory due to their toxic effects and their growing rates of resistance in several

parts of the world [18, 19]. Second line drugs include pentamidine (5) and amphotericin B (6), which are mainly employed in cases where the antimonials are not effective [20]. There are disadvantages associated with the use of these drugs such as their high cost, the need for long-term treatment, the loss of effectiveness due to parasite resistance and severe undesired effects [19, 21, 22]. Recently, miltefosine (7) [23, 24] was approved for the treatment of human visceral leishmaniasis infections, but it is believed that it is teratogenic [25, 26]. Two interesting drugs under clinical trials are sitamaquine (8) [27, 28] and the antibiotic paromomycin (9) (Fig 1) [3, 29]. Extensive studies of new and diverse potential antileishmanial drugs have been made in the last few years.



Figure 1. Chemotherapy of Chagas' disease and leishmaniasis. Chemical structure of current drugs clinically employed for the treatment of these diseases.

However, the problems of drug resistance and the side effects of the chemotherapies used at present have not yet been solved. Consequently, the development of novel, safe and affordable compounds with potent anti-parasitic activity is urgently needed [21, 30].

Quinoline derivatives, mainly reported as antimalarial drugs, are also known to have activity against *Leishmania* spp, trypanosomes, bacteria and tumors [31]. Chloroquine (CQ), one of the most successful antimalarial drugs [32]. showed antimicrobial, antiinflammatory activities and also it is used for the treatment of autoimmune diseases [33-

36]. Some 2-substituted quinolines were shown to be effective in the treatment of cutaneous leishmaniasis and in mice infected with *T. cruzi* [37, 38]. Tipifarnib, another quinoline derivative with anticancer activity, and other analogs have also shown antiparasitic activity [39-41].

Although the mechanism of action of CQ and other aminoquinolines used as antimalarial drugs is not yet fully understood, it is thought to involve an interaction with heme, inhibiting its polymerization to hemozoin [42-46]. The latter is a nontoxic form of heme, which avoids the pro-oxidant properties of the free heme that would lead to the parasite death [31].

In the present study, we report the synthesis of a series of 2- and 3-arylaminoquinoline derivatives **10a-f** and **11a-f** (Fig 2), some of them new compounds, which were biologically evaluated against *T. cruzi* and *L. mexicana*. Finally, the participation of heme in the mechanism of action of these compounds was also investigated.



10a-f: Y = 2-NH, R¹ = 2-F, 3-F, 4-F, 4-Cl, 4-CO₂H, 4-vinyl **11a-f**: Y = 3-NH, R¹ = 2-F, 3-F, 4-F, 4-Cl, 4-CO₂H, 4-vinyl

Figure 2. Aryl 2- and 3-aminoquinoline derivatives. Chemical structure of new synthesized compounds.

2. Results and Discussions

2.1. Synthesis of arylaminoquinolines

It is known that a large number of biologically active compounds have aromatic heterocyclic rings in their structure [47-53]. In particular, quinoline derivatives have shown antibacterial, anti-inflammatory and antiparasitic activity [54-58]. In the course of our program aimed at searching new biologically active nitrogen drugs, we have previously reported the synthesis and biological properties of different compounds [47, 59-61]. In this

case, we have decided to prepare new compounds containing different substituents at quinoline ring.

A series of twelve 2- and 3-arylaminoquinoline derivatives were designed and synthesized considering principles of medicinal chemistry such as ring transformation or inclusion of suitable groups. For example, substitution in the aryl ring by the introduction of halogen atoms, an acid group or an unsaturated side chain could increase potency and improve metabolic stability. Taking this into account we synthesized the compounds **10-11** (Fig 2). Thus, the aryl amino quinolines were straight forwardly prepared via a coupling reaction that involves an aryl boronic acid and an appropriate aromatic amine (Fig 3). Boronic acids are known reagents to form heteroatom-carbon bonds. Likewise, phenylboronic acids are efficient arylating agents. This *N*-arylation methodology may be performed under mild conditions at room temperature in the presence of pyridine and cupric acetate as catalysts. It was observed that the yield of the reaction is remarkably dependent on the nature of the substrate and the substitution on the boronic acid [62-64].



Figure 3. Preparation of 2- and 3-arylaminoquinoline derivatives. Synthetic strategy for obtention of desired compounds.

Starting from commercially available 2- (12) and 3-aminoquinoline (13), the reaction involved their coupling with various substituted phenylboronic acids (14a-f) in dry dichloromethane. Aryl 2- and 3-amino quinolines derivatives, 10a-f and 11a-f respectively, were obtained from good to very good yield (Table 1).

	\mathbf{R}^{1}	2-Arylaminoquinoline (10) Yield (%)	3-Arylaminoquinoline (11) Yield (%)		
a	2-F	66	70		
b	3-F	70	80		
c	4-F	75	85		
d	4-Cl	72	82		
e	$4-CO_2H$	45	50		
f	4-CH=CH ₂	68	75		

Table 1. Yields in the synthesis of new aryl amino quinoline derivatives (10a-f and 11a-f).

The reaction conditions correspond to those described in Materials and Methods.

As it is observed, the reaction yields were influenced by the substituent at the phenyl group and its position in the quinoline ring. The reaction yields with 3-aminoquinoline (**11a-f**) were higher than those with the 2-amino isomer (**10a-f**). Higher yields were obtained with halogen substituents, mainly at the 4 position of the phenyl ring. All compounds were fully characterized through their ¹H and ¹³C NMR, HRMS and IR spectra.

2.2 Anti-Leishmania mexicana activity

The effect of different concentrations of the 2- and 3-arylaminoquinolines (**10a-f** and **11a-f**) on promastigote form of *Leishmania mexicana* growth was studied during 10 days. Geneticin, a well-known antiparasitic agent, was used as a standard inhibitor at a concentration of 50 µg/mL. A remarkable inhibitory effect on *L. mexicana* growth was observed in the case of **10b**, **11b**, **11c** and **11d**. The rest of the tested compounds were active but at a concentration higher than 50 µg/mL. For the lowest dosage of 10 µg/mL, 50% inhibition was detected after 6 days of incubation of **11b** and **11c**. With 25 µg/mL, 25 and 37.5% inhibition respectively was observed after 2 days of exposure and a maximum of 100% was reached after 10 days for both compounds. Figure 4 shows the growth curves of promastigote *L. mexicana* in the presence of compounds **10b**, **11b**, **11c** and **11d**.



Figure 4. Effect of 10b, 11b-d concentration on *Leishmania mexicana* growth rate. Promastigotes were grown at 28 °C for 10 days in the presence of different concentrations of 10b and 11b-d compounds (10-100 μ g/ml). Growth inhibition was estimated by counting the parasites, every 2 days, using a Neubauer chamber. p values < 0.05 (*) and < 0.01 (**) were considered significant.

These four compounds were the most potent against *L. mexicana* with IC₅₀ values of 75.6, 41.9, 41.9 and 98.1 μ M (day 6 of growth) for **10b**, **11b**, **11c** and **11d** respectively, showing better activity than the standard inhibitor geneticin (IC₅₀ 100 μ M) (Table 2).

Comp	Σ IC ₅₀ (μM)				Cytotoxicity
	<i>Lm</i> pro	Тс ері	Tc trypo	Tc amas	CC50 (µM)
10a	> 200	>15	>> 500	>> 50	
10b	75.6 ± 1.0	> 15	811.02 ±	58.4±0.8	> 100
10c	> 200	> 15	>> 500	>> 50	> 100
10d	> 200	> 15	>> 500	>> 50	185
10e	> 200	>> 15	>> 500	>> 50	ND
10f	> 200	>> 15	>> 500	>> 50	ND
11a	> 200	> 15	>> 500	>> 50	ND
11b	41.9± 1.0	13.51 ± 1.20	$419.95~\pm$	$22.8{\pm}~0.9$	> 100
11c	41.9 ± 0.8	11.29 ± 0.63	$616.02 \pm$	10.9 ± 1.2	>> 100
11d	98.1 ± 1.6	12.01 ± 0.18	>> 500	25.8 ± 3.0	> 100
11e	> 200	>> 15	>> 500	>> 50	ND
11f	> 200	>> 15	>> 500	>> 50	ND
Bnz	-	7.56 ± 0.5	38.43 ± 4.2	4.88 ± 0.60	85
Geneticin	100	-	-		-

Table 2. Biological activity of quinoline derivatives against *T. cruzi* (epi-, trypo- and amastigote forms) and *L. mexicana* (promastigote form).

 IC_{50} values were estimated by non-linear regression from the growth inhibition percentage values and log of drug concentration. CC_{50} on Vero cells was calculated by non-linear regression from the percentage of viable cells and the log of drug concentration.

The fluorinated products **11b**, **11c** and **10b** are the most potent, indicating that the presence of the fluorine atom increased the inhibitory activity. Besides, the best compound was the 3-aminoquinoline derivative containing fluorine at 4-position (**11c**). The presence of a fluorine atom could increase the logP value improving cell membrane penetration by a better water/octanol distribution coefficient, resulting in a higher biological activity [65]. Substitution with chlorine at 4-position of the phenyl group (**11d**) afforded a compound which showed similar activity to geneticin.

2.3. Anti-Trypanosoma cruzi activity

In a first screening, all synthesized compounds were evaluated as growth inhibitors against the epimastigote form of *Trypanosoma cruzi* at concentrations ranging from 2.5 μ M to 15 μ M in three independent assays. Benznidazole (Bnz), the drug currently used for treatment of Chagas' disease, was used as a positive control. Halogen containing 3-aminoquinoline derivatives (**11b-d**) were again the most potent compounds showing an inhibitory effect in the same order of benznidazole. The rest of the tested compounds were inactive until concentration of 15 μ M. Figure 5 shows the growth curves that allowed to obtain IC₅₀ values of 13.5, 11.3 and 12.0 μ M for **11b**, **11c** and **11d**, respectively (Table 2).



Figure 5. Effect of 11b, 11c and 11d concentration on the proliferation of *Trypanosoma cruzi* epimastigotes. The parasites were grown at 28 °C for 4 days with different

concentrations (2.5 -15 μ M) of tested compounds. Growth rate was evaluated by counting the parasites using a Neubauer chamber. p values < 0.05 (*) and < 0.01 (**) were considered significant.

The promising finding of the antiparasitic activity of compounds **11b**, **11c** and **11d** against *T. cruzi* epimastigotes prompted us to study their activity against bloodstream stage (trypomastigote form) and intracellular stage (amastigote form) (Table 2). Compounds **11b**, **11c** and **11d** were also those that showed better trypanocidal activity on trypo- and amastigote forms. From Table 2, we conclude that compounds **11b-d** show the highest antiparasitic activity on both *L. mexicana* and *T. cruzi*. In addition, it is important to note that compound **11c** showed IC₅₀ values in the same order of Bzn.

2.4. Cytotoxic activity

The results of cytotoxic activity of compounds **11b**, **11c** and **11d** on Vero cells are also shown in Table 2. For concentrations of these compounds as high as 100 μ M no cytotoxic effect was observed, while for Bnz was of 85 ± 5 μ M.

2.5. Hemin interaction

Trypanosomatids protozoa show a strict requirement of heme-compounds such as hemoglobin, hematin or hemin during their growth [30]. This nutritional characteristic is due to its inability to synthesize heme. Since high levels of hemin and related compounds have an important cytotoxic effect mediated by ROS generation, trypanosomatids present different pathways to counteract the oxidative stress that an excess of heme would generate. In *Plasmodium* spp. this detoxification is carried out by the conversion of heme into hemozoin, a non-toxic polymerized form of heme. In contrast, in *Leishmania* spp. and *Trypanosoma cruzi*, the cytotoxic effect of heme is avoided by its conversion into biliverdin, in which heme oxygenase enzyme would be involved [31].

It is known that quinoline drugs develop their antimalarial activity through the formation of quinoline-heme complexes, which inhibit the heme polymerization into hemozoin, leading to the death of the parasite. According to their structures, our quinoline derivatives could exercise their antiparasitic action by the formation of heme-quinoline

complexes which could inhibit heme detoxification. To prove this possible mode of action, we evaluate the binding of quinolines **11b-d** to heme (Fig 6). These compounds have effectively manifested an interaction with hemin similar than that shown by chloroquine.



Figure 6. Interaction of quinoline derivatives **11b-d** with hemin. After interacting the hemin (7.5 μ M) with different concentrations of **11b-d** quinolines or chloroquine (22.5-90 μ M), the absorption spectra of hemin was recorded. The absorbance ratio at 430 and 400 nm (A430/A400) was used to quantify modifications in the Soret band. p values < 0.05 (*) were considered significant.

2.6. Intracellular redox state

Assuming that quinoline derivatives inhibit heme degradation, its presence in the parasite would generate oxidative stress. Given that the amount of reduced low molecular mass thiols is indicative of the redox state of the cell, we expect that the treatment with the compounds **11b**, **11c** and **11d** affects SH-group levels, because of the formation of oxidant species. For the evaluation of the oxidative stress induced by these quinolines, *T. cruzi* epimastigotes were cultured in the presence of compounds **11b-d** (35 μ M) during 3, 6 and 9 h. Our results (Fig 7A) showed that SH-content remains constant and equal to the untreated parasites (about 52.31 nmol/mg protein), for treatment with the three quinolines for 3 and 6 hours, diminishing between 43%, 35% and 39% for **11b**, **11c** and **11d** compounds,

respectively after 9 h of treatment. The level of SH-groups decrease to counteract oxidative injury induced by treatment with quinolines, confirming the proposed mechanism of action.

Together with these determinations, the intracellular oxidative state was assessed by flow cytometry. As we see in the Figure 7B the behavior of the three quinolines was similar, an increase of about 4 times in the fluorescence intensity of the H₂DCFDA-loaded epimastigotes was observed after 9 h of treatment. This evidences an oxidative stress that persists despite the decrease in the level of thiols, and that would be responsible for the death of the parasite treated with the different quinolines.



Figure 7. Thiol groups content and intracellular oxidative stress during the treatment with 11b-d derivatives. *T. cruzi* epimastigotes, which had been cultured for 4 days, were incubated with 35 μ M **11b-d** compounds during 3, 6 or 9 h. (A) The thiol groups were measured spectrophotometrically after reacting with DTNB. (B) Intracellular oxidative status was evaluated by flow cytometry using probe H₂DCFDA. Gmt/Gmc ratios were calculated as indicated in Materials and Methods.

3. Materials and methods

3.1. General

Chemicals and solvents used in the synthesis were purchased from Merck Argentina and Sigma-Aldrich Argentina and used without further purification. Standard solutions of synthezised compounds were prepared in dimethyl sulphoxide (DMSO) at a final concentration that never exceeded 0.5% DMSO. Hemin, CPRG and H2DCFDA were obtained from Sigma Chem. Co. (Saint Louis, MO, USA). Benznidazole (Bnz) was kindly provided by Roche (Argentina). All other chemicals were of the highest purity commercially available.

3.2. General procedure for the synthesis of arylaminoquinolines

A flask is loaded with 2-amino or 3-aminoquinoline (1 mmol), cupric acetate (1 mmol), the corresponding arylboronic acid (2 mmol) and 4Å molecular sieves. The reaction mixture is diluted with dichloromethane (5.0 mL) and pyridine (2 mmoL) is added. After stirring the heterogeneous reaction mixture for 18 h at 25 °C under nitrogen atmosphere, the resulting slurry is filtered and the product is isolated from the organic filtrate by column chromatography (silica gel) employing mixtures of hexane-EtOAc as eluent (7:3 - 2:3). To monitor the reaction progress aliquots were withdrawn and analyzed by TLC performed on commercial 0.2 mm aluminum-coated silica gel plates (F254), using EtOAc:hexane 3:2 as developing solvent and visualized by 254 nm UV or immersion in an aqueous solution of (NH₄)₆Mo₇O₂₄.4H₂O (0.04 M), Ce(SO₄)₂ (0.003 M) in concentrated H₂SO₄ (10%). ¹H NMR and ¹³C NMR spectra were recorded at room temperature in CDCl₃ as solvent using a Bruker AM-500 NMR instrument operating at 500.14 MHz and 125.76 MHz for ¹H and ¹³C respectively. The ¹H NMR spectra are referenced with respect to the residual CHCl₃ proton of the solvent CDCl₃ at $\delta = 7.26$ ppm. Coupling constants are reported in Hertz (Hz). ¹³C NMR spectra were proton decoupled and are referenced to the middle peak of the solvent CDCl₃ at $\delta = 77.0$ ppm. Splitting patterns are designated as: s, singlet; d, doublet; t, triplet; q, quadruplet; qn, quintet; dd, double doublet, etc. High Resolution Mass Spectrometry was recorded with Thermo Scientific EM/DSQ II - DIP. The results were within $\pm 0.02\%$ of the theoretical values.

1. 2-(2-Fluor-phenylamino) quinoline (**10a**). Yield 66% of pure compound as a yellow oil. ¹H NMR (CDCl₃) δ 6.60 (m, 2H, H-14, H-16), 6.97 (d, *J* = 8.5 Hz, 1H, H-3), 7.16 (m, 2 H, H-13, H-15), 7.33 (dt, *J*₁ = 1.2 Hz, *J*₂ = 8.0 Hz, 1 H, H-6), 7.61 (dt, *J*₁ = 1.1 Hz, *J*₂ = 8.2 Hz, 1H, H-7), 7.67 (dd, *J*₁ = 1.1 Hz, *J*₂ = 8.0 Hz, 1 H, H-8), 7.83 (dd, *J*₁ = 1.1 Hz, *J*₂ = 8.0 Hz, 1 H, H-5), 7.97 (d, *J* = 8.4 Hz, 1H, H-4); ¹³C NMR (CDCl₃) δ 111.5 (C-3), 115.6 (d, *J* = 23.4 Hz, C-13), 122.3 (d, *J* = 9.6 Hz, C-14, C-16), 123.9 (C-8), 124.5 (C-10), 126.8 (C-15), 127.7 (C-5, C-6), 130.4 (C-7), 130.5 (d, *J* = 9.6 Hz, C-11), 138.5 (C-4), 147.3 (C-9), 153.9 (C-2), 164.7 (d, *J* = 244.3 Hz, C-12); HRMS: [M + Na]⁺ Calcd. C₁₅H₁₁FN₂Na 261.0804. Found: C₁₅H₁₁FN₂Na 261.0812.

2. 2-(3-Fluor-phenylamino) quinoline (10b). Yield 70% of pure compound as a light yellow oil. ¹H NMR (CDCl₃) δ 6.77 (ddd, $J_1 = 2.3$ Hz, $J_2 = 8.3$ Hz, 1H, H-14), 6.98 (d, J = 8.6 Hz, 1H, H-3), 7.28 (m, 2 H, H-15, H-16), 7.35 (dt, $J_1 = 1.1$ Hz, $J_2 = 8.0$ Hz, 1 H, H-6), 7.62 (m, 2 H, H-7, H-12), 7.67 (dd, $J_1 = 1.3$ Hz, $J_2 = 7.9$ Hz, 1 H, H-8), 7.84 (dd, $J_1 = 1.2$ Hz, $J_2 = 8.0$ Hz, 1 H, H-5), 7.98 (d, J = 8.6 Hz, 1H, H-4); ¹³C NMR (CDCl₃) δ 103.2 (d, J = 24.4 Hz, C-12), 109.5 (d, J = 22.4 Hz, C-14), 111.2 (C-3), 111.9 (C-16), 115.4 (d, J = 9.5 Hz, C-15), 123.6 (C-8, C-10), 127.5 (C-5, C-6), 130.1 (C-7), 130.4 (d, J = 9.6 Hz, C-11), 138.2 (C-4), 147.1 (C-9), 153.6 (C-2), 162.4 (d, J = 244.3 Hz, C-13); HRMS: [M + Na]⁺ Calcd. C₁₅H₁₁FN₂Na 261.0804. Found: C₁₅H₁₁FN₂Na 261.0799.

3. 2-(**4-Fluor-phenylamino**) **quinoline** (**10c**). Yield 75% of pure compound as a yellow oil. ¹H NMR (CDCl₃) δ 6.89 (d, J = 8.2 Hz, 1H, H-3), 7.06 (d, J = 8.6 Hz, 2 H, H-13, H-15), 7.31 (dt, $J_1 = 1.2$ Hz, $J_2 = 7.6$ Hz, 1 H, H-6), 7.55 (d, J = 8.7 Hz, 2 H, H-12, H-16), 7.60 (dt, $J_1 = 1.5$ Hz, $J_2 = 7.5$ Hz, 1 H, H-7), 7.64 (dd, $J_1 = 1.0$ Hz, $J_2 = 7.8$ Hz, 1 H, H-8), 7.77 (dd, $J_1 = 1.0$ Hz, $J_2 = 7.6$ Hz, 1 H, H-5), 7.92 (d, J = 8.0 Hz, 1H, H-4); ¹³C NMR (CDCl₃) δ 111.4 (C-3), 115.8 (d, J = 22.5 Hz, C-13, C-15), 122.6 (d, J = 7.8 Hz, C-12, C-16), 122.7 (C-8), 123.2 (C-10), 126.6 (C-6), 127.4 (C-5), 129.9 (C-7), 136.1 (C-11), 137.9 (C-4), 147.5 (C-9), 154.4 (C-2), 160.0 (d, J = 242.6 Hz, C-14); HRMS: [M + Na]⁺ Calcd. C₁₅H₁₁FN₂Na 261.0804. Found: C₁₅H₁₁FN₂Na 261.0810.

4. 2-(4-Chloro-phenylamino) quinoline (10d). Yield 72% of pure compound as a light yellow oil. ¹H NMR (CDCl₃) δ 6.94 (d, *J* = 9.0 Hz, 1H, H-3), 7.32-7.33 (m, 3 H, H-6, H-13, H-15), 7.52 (d, *J* = 7.8 Hz, 2 H, H-12, H-16), 7.61 (dt, *J*₁ = 1.5 Hz, *J*₂ = 7.1 Hz, 1 H, H-7), 7.66 (dd, *J*₁ = 1.5 Hz, *J*₂ = 8.1 Hz, 1 H, H-8), 7.79 (dd, *J*₁ = 1.3 Hz, *J*₂ = 8.4 Hz, 1 H, H-5), 7.95 (d, *J* = 8.9 Hz, 1H, H-4); ¹³C NMR (CDCl₃) δ 111.6 (C-3), 116.7 (C-12, C-16), 121.8 (C-8), 123.5 (C-6), 124.1 (C-10), 126.4 (C-14), 127.5 (C-5), 129.5 (C-12, C-16), 130.1 (C-7), 136.7 (C-11), 138.2 (C-4), 149.1 (C-9), 154.6 (C-2); HRMS: [M + Na]⁺ Calcd. C₁₅H₁₁ClN₂Na 277.0508. Found: C₁₅H₁₁ClN₂Na 277.0512.

5. 2-(**4-Carboxy-phenylamino**) **quinoline** (**10e**). Yield 45% of pure compound as a light yellow oil. ¹H NMR (CDCl₃) δ 6.88 (d, *J* = 8.0 Hz, 1H, H-3), 7.30 (dt, *J*₁ = 1.2 Hz, *J*₂ = 7.6 Hz, 1 H, H-6), 7.59 (dt, *J*₁ = 1.4 Hz, *J*₂ = 7.6 Hz, 1 H, H-7), 7.64 (dd, *J*₁ = 1.2 Hz, *J*₂ = 7.7 Hz, 1 H, H-8), 7.70 (d, *J* = 8.6 Hz, 2 H, H-12, H-16), 7.77 (dd, *J*₁ = 1.1 Hz, *J*₂ = 7.6 Hz, 1 H, H-5), 7.92 (d, *J* = 7.9 Hz, 1H, H-4), 8.00 (d, *J* = 8.6 Hz, 2 H, H-13, H-15); ¹³C NMR (CDCl₃) δ 112.2 (C-3), 116.7 (C-12, C-16), 123.5 (C-8, C-14), 124.0 (C-10), 127.4 (C-6), 128.2 (C-5), 130.7 (C-7), 132.9 (C-13, C-15), 138.7 (C-4); 141.7 (C-11); 148.3 (C-9); 155.2 (C-2), 165.2 (-CO₂H); HRMS: [M + Na]⁺ Calcd. C₁₆H₁₂N₂NaO₂ 287.0796. Found: C₁₆H₁₂N₂NaO₂ 287.0790.

6. 2-(4-Vinyl-phenylamino) quinoline (10f). Yield 68% of pure compound as a light orange oil. ¹H NMR (CDCl₃) δ 5.16 (dd, $J_1 = 0.9$ Hz, $J_2 = 10.9$ Hz, 1H, H-18a), 5.66 (dd, $J_1 = 0.9$ Hz, $J_2 = 17.5$ Hz, 1H, H-17b), 6.67 (dd, $J_1 = 10.9$ Hz, $J_2 = 17.5$ Hz, 1H, H-17b), 6.91 (d, J = 8.9 Hz, 1H, H-3), 7.30 (m, 3 H, H-6, H-13, H-15), 7.50 (d, J = 7.9 Hz, 2 H, H-12, H-16), 7.58 (dt, $J_1 = 1.6$ Hz, $J_2 = 7.0$ Hz, 1 H, H-7b), 7.63 (dd, $J_1 = 1.5$ Hz, 2 = 8.0 Hz, 1 H, H-8b), 7.77 (dd, $J_1 = 1.2$ Hz, $J_2 = 8.5$ Hz, 1 H, H-5b), 7.93 (d, J = 9.0 Hz, 1H, H-4b); ¹³C NMR (CDCl₃) δ 110.0 (C-3), 112.7 (=CH2), 116.7 (C-12, C-16), 120.21 (C-8), 121.9 (C-6), 122.5 (C-10), 124.8 (C-14), 125.9 (C-5), 127.9 (C-13, C-15), 128.5 (C-7), 135.1 (C-11), 136.0 (CH=), 136.6 (C-4), 147.5 (C-9), 153.0 (C-2); HRMS: [M + Na]⁺ Calcd. C₁₇H₁₄N₂Na 269.1055. Found: C₁₇H₁₄N₂Na 269.1053.

7. 3-(**2**-Fluor-phenylamino) quinoline (11a). Yield 70% of pure compound as a yellow oil. ¹H NMR (CDCl₃) δ 7.05 (m, 4H, H-13, H-14, H-15, H-16), 7.39 (dt, $J_1 = 1.3$ Hz, $J_2 = 7.9$ Hz, 1 H, H-7), 7.49 (dt, $J_1 = 1.1$ Hz, $J_2 = 8.0$ Hz, 1H, H-6), 7.54 (dd, $J_1 = 1.1$ Hz, $J_2 = 8.0$ Hz, 1 H, H-6), 7.54 (dd, $J_1 = 1.1$ Hz, $J_2 = 8.0$ Hz, 1 H, H-6), 7.98 (d, J = 8.4 Hz, 1H, H-8), 8.64 (s, 1H, H-2); ¹³C NMR (CDCl₃) δ 114.3 (C-4), 114.9 (d, J = 23.4 Hz, C-13), 122.7 (d, J = 8.5 Hz, C-16), 123.9 (d, J = 8.3 Hz, C-14), 124.9 (C-5), 125.0 (C-6), 125.4 (d, J = 2.5 Hz, C-15), 125.8 (C-7), 127.4 (C-10), 127.6 (C-8), 129.5 (d, J = 26 Hz, C-11), 136.4 (C-9), 142.1 (C-3), 143.0 (C-2), 157.4 (d, J = 244.0 Hz, C-12); HRMS: [M + Na]⁺ Calcd. C₁₅H₁₁FN₂Na 261.0804. Found: C₁₅H₁₁FN₂Na 261.0809.

8. 3-(**3**-Fluor-phenylamino) quinoline (11b). Yield 80% of pure compound as a yellow oil. ¹H NMR (CDCl₃) δ 7.07 (ddd, $J_1 = 2.1$ Hz, $J_2 = 8.2$ Hz, 1H, H-14), 7.16 (m, 2 H, H-15, H-16), 7.37 (dt, $J_1 = 1.0$ Hz, $J_2 = 8.2$ Hz, 1 H, H-12), 7.47 (dt, $J_1 = 1.2$ Hz, $J_2 = 8.0$ Hz, 1 H, H-7), 7.50 (dt, $J_1 = 1.1$ Hz, $J_2 = 8.1$ Hz, 1H, H-6), 7.54 (dd, $J_1 = 1.2$ Hz, $J_2 = 8.1$ Hz, 1 H, H-4), 7.61 (dd, $J_1 = 1.0$ Hz, $J_2 = 8.0$ Hz, 1 H, H-5), 7.99 (d, J = 8.3 Hz, 1H, H-8), 8.65 (s, 1H, H-2); ¹³C NMR (CDCl₃) δ 108.5 (d, J = 24.2 Hz, C-12), 111.5 (d, J = 22.7 Hz, C-14), 111.2 (C-3), 113.7 (d, J = 2.5 Hz, C-16), 115.7 (C-4), 117.2 (d, J = 8.2 Hz, C-15), 126.4 (C-5, C-6), 127.2 (C-7), 128.8 (C-10), 129.0 (C-8), 130.8 (d, J = 8.0 Hz, C-11), 137.8 (C-9), 143.4 (C-3), 144.4 (C-2), 158.8 (d, J = 242.0 Hz, C-13); HRMS: [M + Na]⁺ Calcd. C₁₅H₁₁FN₂Na 261.0804. Found: C₁₅H₁₁FN₂Na 261.0810.

9. 3-(**4**-Fluor-phenylamino) quinoline (11c). Yield 85% of pure compound as light yellow colorless oil. ¹H NMR (CDCl₃) δ 5.89 (ws, 1H, NH), 7.07 (t, J = 8.2 Hz, 2H, H-13, H-15), 7.16 (dd, $J_1 = 4.7$ Hz, $J_2 = 8.4$ Hz, 2 H, H-12, H-16), 7.45 (dt, $J_1 = 1.3$ Hz, $J_2 = 7.8$ Hz, 1 H, H-7), 7.51 (dt, $J_1 = 1.4$ Hz, $J_2 = 6.7$ Hz, 1 H, H-6), 7.57 (d, J = 2.6 Hz, 1 H, H-4), 7.61 (dd, $J_1 = 1.5$ Hz, $J_2 = 8.0$ Hz, 1 H, H-5), 7.99 (d, J = 8.2 Hz, 1H, H-8), 8.65 (s, 1H, H-2); ¹³C NMR (CDCl₃) δ 115.7 (C-4), 116.3 (d, J = 22.7 Hz, C-13, C-15), 121.5 (d, J = 7.8 Hz, C-12, C-16), 126.3 (C-5), 126.4 (C-6), 127.2 (C-7), 128.8 (C-10), 129.0 (C-78), 137.5 (d, J = 2.6 Hz, C-11), 137.8 (C-9), 143.5 (C-3), 144.4 (C-2), 158.7 (d, J = 242.0 Hz, C-14); HRMS: [M + Na]⁺ Calcd. C₁₅H₁₁FN₂Na 261.0804. Found: C₁₅H₁₁FN₂Na 261.0808.

10. 3-(**4**-**Chloro-phenylamino**) **quinoline** (**11d**). Yield 82% of pure compound as a light yellow oil. ¹H NMR (CDCl₃) δ 6.02 (ws, 1H, NH), 7.11 (d, *J* = 8.7 Hz, 2H, H-13, H-15), 7.30 (d, *J* = 8.7 Hz, 2 H, H-12, H-16), 7.49 (dt, *J*₁ = 1.6 Hz, *J*₂ = 7.0 Hz, 1 H, H-7), 7.51 (dt, *J*₁ = 1.6 Hz, *J*₂ = 6.9 Hz, 1 H, H-6), 7.66 (dd, *J*₁ = 1.8 Hz, *J*₂ = 8.2 Hz, 1 H, H-5), 7.69 (d, *J* = 2.7 Hz, 1 H, H-4), 8.01 (d, *J* = 8.3 Hz, 1H, H-8), 8.69 (d, *J* = 2.7 Hz, 1H, H-2); ¹³C NMR (CDCl₃) δ 117.6 (C-4), 119.7 (C-12, C-16), 126.5 (C-5), 126.9 (C-6), 127.1 (C-14), 127.3 (C-7), 128.7 (C-10), 128.8 (C-8), 129.6 (C-13, C-15), 136.6 (C-11), 140.5 (C-9), 143.6 (C-3), 144.8 (C-2); HRMS: [M + Na]⁺ Calcd. C₁₅H₁₁ClN₂Na 277.0508. Found: C₁₅H₁₁ClN₂Na 277.0505.

11. 3-(**4**-**Carboxy-phenylamino**) **quinoline** (**11e**). Yield 50% of pure compound as a yellow oil. ¹H NMR (CDCl₃) δ 6.00 (ws, 1H, NH), 7.47 (dt, $J_1 = 1.6$ Hz, $J_2 = 7.0$ Hz, 1 H, H-7), 7.49 (dt, $J_1 = 1.6$ Hz, $J_2 = 6.9$ Hz, 1 H, H-6), 7.63 (dd, $J_1 = 1.8$ Hz, $J_2 = 8.2$ Hz, 1 H, H-5), 7.67 (d, J = 2.7 Hz, 1 H, H-4), 7.75 (d, J = 8.7 Hz, 2H, H-13, H-15), 7.91 (d, J = 8.7 Hz, 2 H, H-12, H-16), 8.00 (d, J = 8.3 Hz, 1H, H-8), 8.67 (d, J = 2.7 Hz, 1H, H-2); ¹³C NMR (CDCl₃) δ 116.9 (C-12, C-16), 117.8 (C-4), 125.2 (C-14), 126.6 (C-5), 127.0 (C-6), 127.5 (C-7), 128.9 (C-10), 129.0 (C-8), 130.4 (C-13, C-15), 140.4 (C-11), 140.6 (C-9), 143.7 (C-3), 145.0 (C-2), 167.8 (-CO₂H); HRMS: [M + Na]⁺ Calcd. C₁₆H₁₂N₂NaO₂ 287.0796. Found: C₁₆H₁₂N₂NaO₂ 287.0801.

12. 3-(**4**-Vinyl-phenylamino) quinoline (11f). Yield 75% of pure compound as a dark yellow oil. ¹H NMR (CDCl₃) δ 5.16 (dd, $J_1 = 0.8$ Hz, $J_2 = 10.9$ Hz, 1H, H-18a), 5.66 (dd, $J_1 = 0.8$ Hz, $J_2 = 17.5$ Hz, 1H, H-18b), 6.04 (ws, 1H, NH), 6.67 (dd, $J_1 = 10.9$ Hz, $J_2 = 17.6$ Hz, 1H, H-17), 7.12 (d, J = 8.5 Hz, 2 H, H-6, H-12, H-16), 7.38 (d, J = 8.5 Hz, 2 H, H-13, H-15), 7.46 (dt, $J_1 = 1.6$ Hz, $J_2 = 6.8$ Hz, 1 H, H-7), 7.51 (dt, $J_1 = 1.6$ Hz, $J_2 = 6.8$ Hz, 1 H, H-6), 7.62 (dd, $J_1 = 1.6$ Hz, $J_2 = 8.0$ Hz, 1 H, H-5), 7.72 (d, J = 2.8 Hz, 1H, H-4), 7.99 (dd, $J_1 = 1.2$ Hz, $J_2 = 8.6$ Hz, 1 H, H-8), 8.68 (d, J = 2.7 Hz, 1H, H-2); ¹³C NMR (CDCl₃) δ 112.1 (=CH2), 117.5 (C-4), 118.3 (C-12, C-16), 125.0 (C-14), 126.5 (C-5), 126.7 (C-6), 127.2 (C-7), 127.5 (C-13, C-15), 128.9 (C-8), 131.9 (C-10), 136.1 (CH=), 136.7 (C-11), 141.4 (C-9), 143.5 (C-3), 144.9 (C-2); HRMS: [M + Na]⁺ Calcd. C₁₇H₁₄N₂Na 269.1055. Found: C₁₇H₁₄N₂Na 269.1061.

3.3. Parasites

Leishmania mexicana promastigotes were grown in a culture medium containing 3.3% brain-heart infusion (Difco); 0.3% tryptose (Difco); 0.3% disodium phosphate; 0.04% potassium chloride; 0.03% dextrose. pH was about 7.5, without the need for adjustment. After sterilization penicillin (100 IU mL⁻¹), streptomycin (100 μ g mL⁻¹), hemin (20 μ g mL⁻¹) and 20% v/v heat-inactivated fetal calf serum was added. Cultures were performed at 28 °C in 15 mL cylindrical flasks with screw caps, containing 2 mL of medium. Shaking was performed manually, twice a day. All cultures were started with inocula taken from exponential growth parasite in the same medium.

Trypanosoma cruzi epimastigotes (Tulahuén strain) were grown, in the same liquid medium as the composition mentioned for *L. mexicana* added with hemin (20 μ g mL⁻¹), under the conditions described previously [66]. At the time of inoculating the parasite this medium was supplemented with penicillin (100 IU mL⁻¹), streptomycin (100 μ g mL⁻¹) and 10% v/v heat-inactivated fetal calf. Cultures were routinely maintained in the exponential phase by weekly passages at 28 °C. Bloodstream trypomastigotes were obtained from infected CF1 mice by cardiac puncture, at the peak of parasitemia on day 15 post-infection. Trypomastigotes were routinely maintained by infecting 21 days-old CF1 mice. Inbred CF1 male mice were nursed at Facultad de Medicina, Universidad de Buenos Aires. Animals were handled in accordance with guidelines established by the Animal Care and Use Committee of the Argentine Association of Specialists in Laboratory Animals (AADEALC).

3.4. In vitro assays for anti-leishmanial activity

The susceptibilities of the promastigote form of *Leishmania* to synthetic compounds were tested by culturing them in cell-free medium at 28 °C. Growth experiments with promastigotes of L. mexicana were initiated with 5 x 10^6 parasites/mL, and the inhibitors were added at 10, 25, 50 and 100 µg/mL from concentrated stock solutions in DMSO at this moment.

A solution of 50 μ g/mL of geneticin was used as a positive control. Parasite grow was evaluated by reading parasites in a Neubauer chamber every two day for ten days. DMSO concentration in the cultures (0.1%) did not interfere with parasite growth or morphology.

The IC₅₀ (50 % inhibitory concentration) was determined by non-linear regression analysis of the data (Sigma Plot 12 Software).

3.5. In vitro assays for anti-trypanocidal activity

To evaluate the growth inhibition of epimastigotes, $0.75 - 1.25 \times 10^7$ parasites/ml were cultured in the presence of 2.5 to 15 µM of the compounds or Bnz (used as positive control) for 4 days. Cells growth was assessed by counting the number of cells per ml of culture using a Neubauer chamber and was expressed as cellular density (CD). The percentage of inhibition (%I) was calculated as: %I = {1-[(CD_{4t}-CD₀)/(CD_{4c}-CD₀)]} x 100, where the different CDs represent the cellular density of: CD_{4t}, treated parasites on day 4; CD₀, parasites on day 0; and CD_{4c}, untreated parasites (control) on day 4.

The trypanocidal effects of different quinolines and Bnz were also tested on bloodstream trypomastigotes according to a standard WHO protocol slightly modified [67]. Briefly, mouse blood containing trypomastigotes (1.5 x 10⁶ parasites/ml) were seeded (150 µl/well) in duplicate in a 96-well plate and treated with different concentrations of each compound (0.45 to 450 µM) or Bnz (0.38 to 380 µM). Plates were incubated for 24 h, and surviving parasites were counted in a Neubauer chamber. The results were expressed as the percentage of lysed parasites (%L) relative to the number of parasites in the control: %L = $[1 - (CD_t/(CD_c)] \times 100$, where CD_t and CD_c represents the cellular density of treated and untreated parasites, respectively.

For analysis of amastigotes, J774 macrophages line was infected with transfected bloodstream trypomastigotes, which expressing the ??-galactosidase gene, using a parasite: cell ratio of 10:1. Then 24 h, the cultures were washed and different concentration of each compound (2 to 50 μ M) was added in fresh RPMI medium without phenol red. After 7 days, the galactosidase activity was measured employing CPRG as substrate and recording absorbance at 570 nm in a Microplate Reader (Bio-Rad Laboratories). Blanks employing uninfected cell were performed. The percentage of inhibition was calculated as %?? =[1]×100, where ?? represents the mean ??570 value recorded for ??it, treated infected cells; ??nit, treated noninfected cells; ??ic, untreated infected cells; and ??nic, untreated noninfected cells. To calculate the IC₅₀ (50% inhibitory concentration) for each parasite form, I% or %L values were plotted against the logarithm (log) of drug concentration (μ M)

and fitted to a sigmoidal curve determined by a non-linear regression (Sigma Plot 12 software).

3.6. Cytotoxicity assay

Cytotoxic activity was evaluated in vitro, using Vero cells. The cells were cultured in RPMI medium at 37 °C 5% CO₂. Cells, 9 x 10⁵ cell/mL were seeded in a 24-well plate and then 24 h different concentrations of compounds (5 - 100 μ M) or Bnz (5 - 200 μ M) were added. After 48 h of incubation, cells were washed twice with PBS and 3-(4,5dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) was added at a final concentration of 0.5 mg/ml. Plates were incubated for 1h at 37 °C. Finally, blue precipitates were dissolved in 0.5 mL DMSO and were read on a plate reader (Spectra CountTM BS 10001) at a wavelength of 570 nm. Values from blank wells containing only medium and reagents were subtracted from the values of the samples. All MTT assays were repeated two times by using three samples per assay. The CC₅₀ (50% cytotoxic concentration) was calculated by non-linear regression analysis (sigmoidal curve) from the percentage of viable cells and the decimal logarithm (log) of drug concentration.

3.7. Hemin binding assay

The interaction of the quinolines with the hemin (which is in non-polymerization conditions) was evaluated spectrophotometrically by monitoring the hemin Soret band. The assay system contained 0.23 M sodium phosphate buffer pH 7.4, 1% SDS, 7,5 μ M hemin and different concentrations of the compounds to be tested (22,5 to 90 μ M). The absorption spectra of hemin was measured using a Hewlett-Packard 8452-Diode Array spectrophotometer. The absorbance ratio at 430 and 400 nm (A₄₃₀/A₄₀₀) was used to quantify modifications in the Soret band. Chloroquine was used as positive control.

3.8. Reduced thiol groups assay

T. cruzi epimastigotes growing in logarithmic phase were incubated with **11b-d** (35 μ M) during 3, 6 or 9 h. Treated-parasites were harvested by centrifugation at 12,000 xg for 10 min, washed once, and resuspended in 50 mM sodium phosphate buffer pH 7.4. Cells in suspension were disrupted by sonication in an MSE Soniprep 150 ultrasonic disintegrator

for 45 s. The resulting homogenate (H) was employing for measuring the level of SHgroups and protein content. To quantify thiols, the H homogenate was treated with 50% trichloroacetic acid to a final concentration of 5%, the precipitated protein was discarded by centrifugation and reduced thiols measured in the supernatant using the chromogenic compound 5,5'-dithiobis-2-nitrobenzoate (DTNB), as already described [46]. Protein concentration was determined according to the method described by Lowry et al [68], and these values were considered to express the content of SH-groups per mg of protein.

3.9. Intracellular oxidative activity assay

The induction of intracellular oxidative stress was assessed using the oxidantsensitive fluorescent probe H₂DCFDA. *T. cruzi* epimastigotes growing in logarithmic phase were incubated with **11b-d** (35 μ M) during 3, 6 or 9 h. Treated-parasites were harvested and stained for 45 min in the dark with 10 μ M H₂DCFDA at 37 °C. As positive control, parasites were treated with 0.2 mM H₂O₂. The fluorescence intensity of dichlorofluorescein (DCF) in cells was then analyzed in a Becton Dickinson FACScalibur flow cytometer with an excitation wavelength of 480 nm and an emission wavelength of 530 nm. Results were expressed by the ratio G_{mt}/G_{mc}, where G_{mt} and G_{mc} correspond to the geometric mean of histograms obtained for treated and untreated (control) cells respectively. Flow cytometry data were analysed employing WinMDI 2.9 software.

3.10. Statistical analysis

Results are representative of three to four separate experiments, performed in duplicate or triplicate. Data are expressed as means \pm standard errors of the mean (SEMs). The significance of differences was evaluated using Student's t test, or One-way ANOVA; p values < 0.05 (*) and < 0.01 (**) were considered significant.

4. Conclusions

This work describes the synthesis and biological evaluation of arylaminoquinoline derivatives, some of them new compounds, which were fully characterized by spectroscopic methods. They were obtained in good yield with a simple reaction with affordable starting materials. All compounds were evaluated against promastigote

Leishmania mexicana and epimastigote, trypomastigote and amastigote forms of *Trypanosoma cruzi*. Fluorine and chlorine derivatives of 3-aminoquinoline (**11b-c**) showed to be very effective as antiparasitic agents on *L. mexicana* and *T. cruzi* exhibiting an efficacy comparable to that of presented by with IC_{50} values of the same order than used control compounds. These compounds were capable to inhibit the degradation of heme, inducing intracellular oxidative damage, which is not countered by the antioxidative defense system of the parasite. In addition, they did not showed citotoxicity, therefore these compounds offer excellent perspective as potential drugs for chemotherapy of neglected diseases as trypanosomiasis and leishmaniasis.

Supporting Information: Spectral data for compounds **10-11**, associated with this article are supplied (for reviewers' information only).

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Graphical Abstract



Highlights:

- New 2- and 3-arylaminoquinolines were prepared and totally spectroscopy characterized.
- Fluorine derivatives showed a remarkable activity against *L. mexicana* and *T. cruzi*.

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- Heme polymerization inhibition is the action mechanism proposed.
- All of synthesized derivatives did not showed citotoxicity.