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

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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 IC_{50} values of 41.9 µM. The IC_{50} 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.

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 and HIV co-infection has been increased due to appearance of visceral 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].

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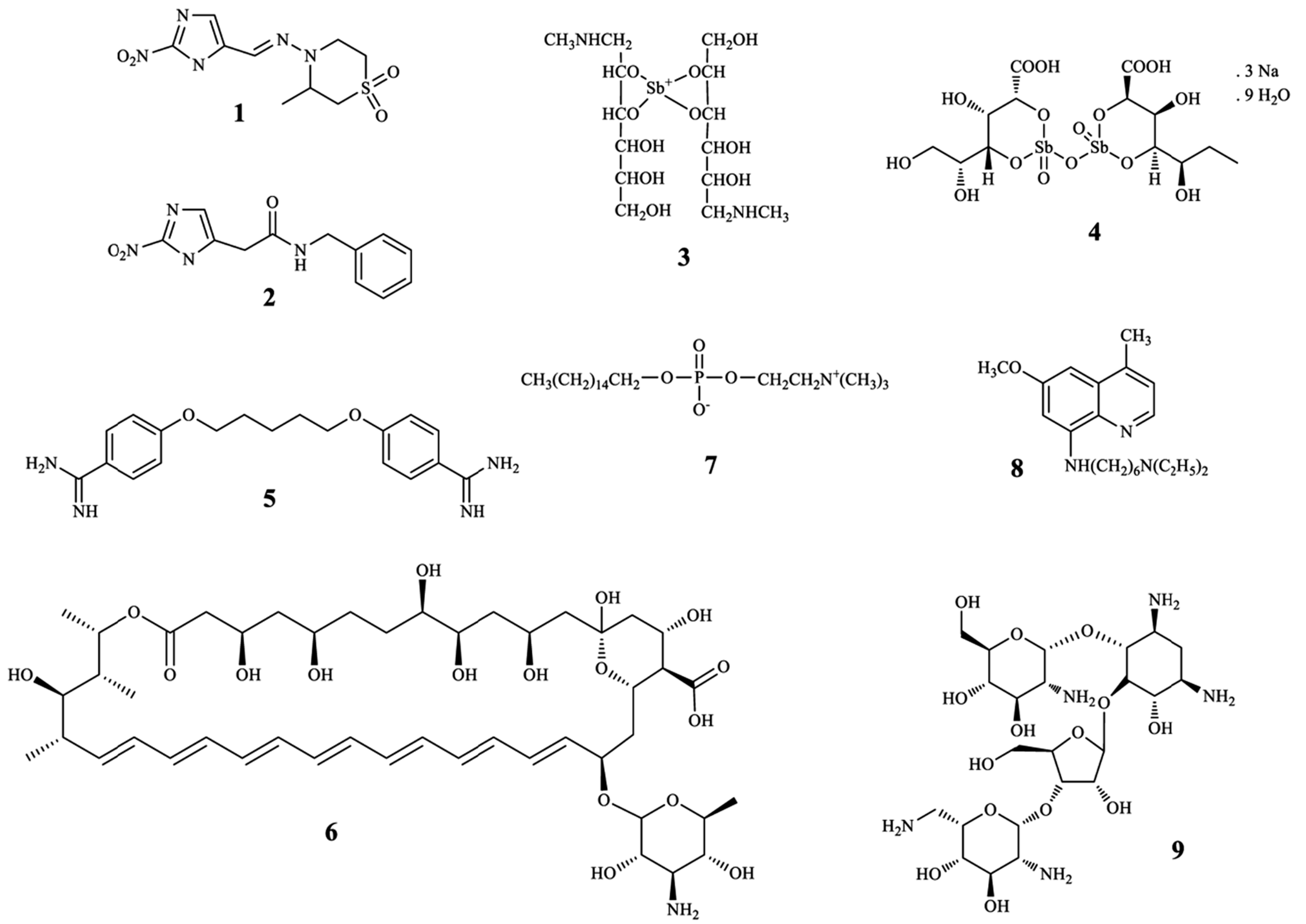


Fig. 1. Chemotherapy of Chagas'disease and leishmaniasis. Chemical structure of current drugs clinically employed for the treatment of these diseases.

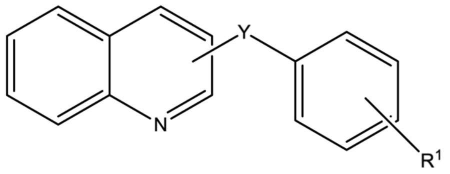
The chemotherapy for leishmaniasis has been based for over 60 years on the use of pentavalent antimonial drugs being meglumine antimoniate (Glucantime) (**3**) or sodium stibogluconate (Pentostam) (**4**) the first-choice drugs (Fig. 1) [17]. However, these drugs are not satisfactory due to their toxic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

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, anti-inflammatory 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, R^1 = 2-F, 3-F, 4-F, 4-Cl, 4-CO_2H, 4-vinyl
11a-f: Y = 3-NH, R^1 = 2-F, 3-F, 4-F, 4-Cl, 4-CO_2H, 4-vinyl

Fig. 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].

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).

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 ^{13}C NMR, HRMS and IR spectra.

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

	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

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

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. Fig. 4 shows the growth curves of promastigote *L. mexicana* in the presence of compounds **10b**, **11b**, **11c** and **11d**.

These four compounds were the most potent against *L. mexicana* with IC_{50} values of 75.6, 41.9, 41.9 and 98.1 µM (day 6 of growth) for **10b**, **11b**, **11c** and **11d** respectively, showing better activity than the standard inhibitor geneticin (IC_{50} 100 µM) (Table 2).

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 µM to 15 µM 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 µM. Fig. 5 shows the growth curves that allowed to obtain IC_{50} values of 13.5, 11.3 and 12.0 µM for **11b**, **11c** and **11d**, respectively (Table 2).

The promising finding of the antiparasitic activity of compounds **11b**, **11c** and **11d** against *T. cruzi* epimastigotes prompted us to study

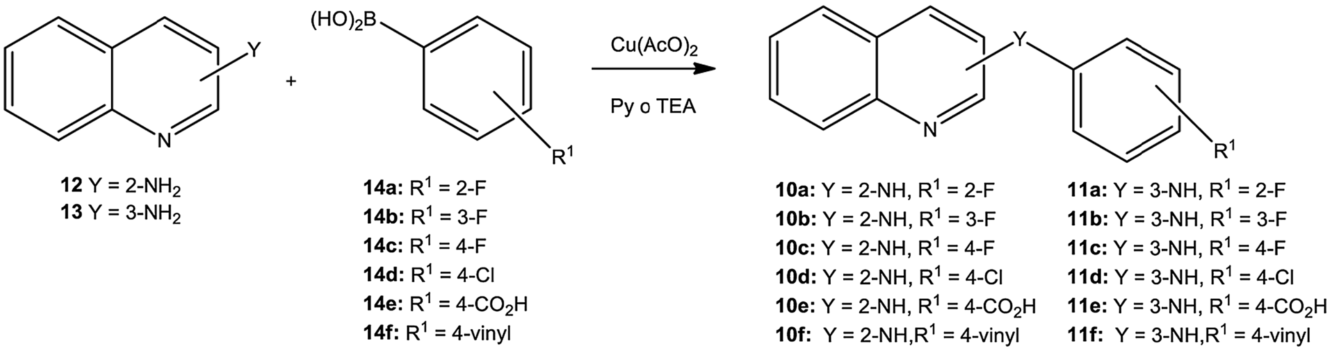


Fig. 3. Preparation of 2- and 3-arylaminoquinoline derivatives. Synthetic strategy for obtention of desired compounds.

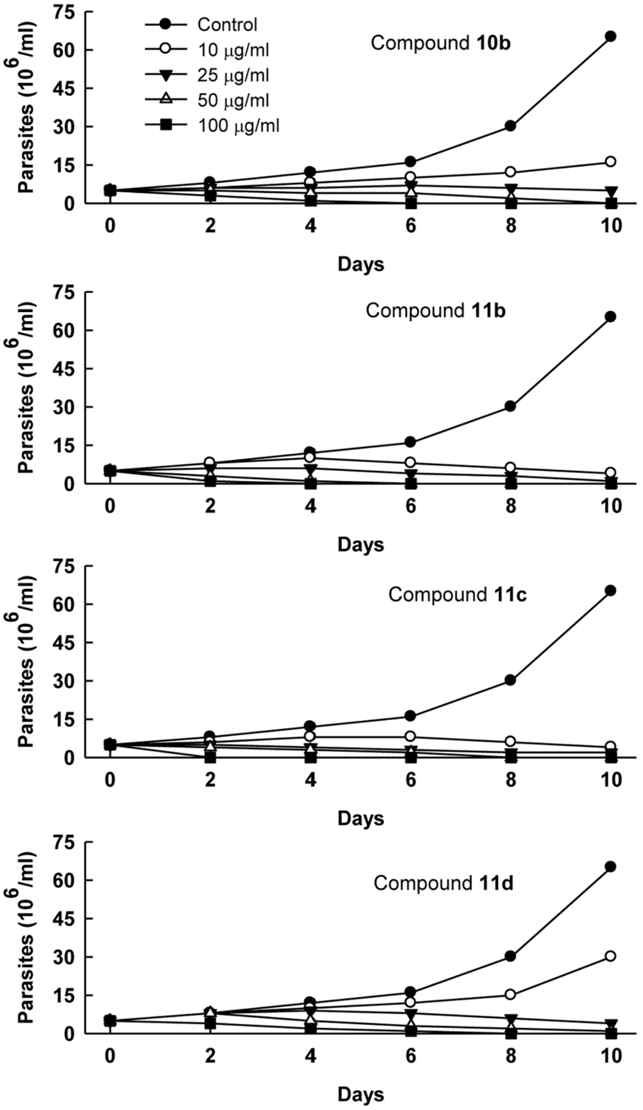


Fig. 4. Effect of **10b**, **11b–d** concentration on *Leishmania mexicana* growth rate. Promastigotes were grown at 28 °C for 10 days in the presence of different concentrations of **10b** and **11b–d** compounds (10–100 μg/ml). Growth inhibition was estimated by counting the parasites, every 2 days,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

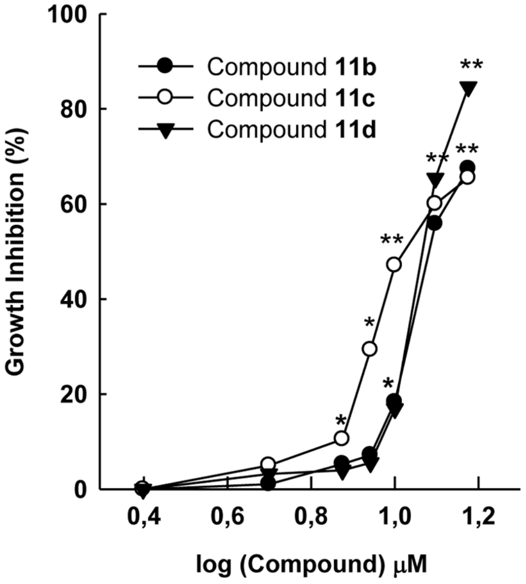


Fig. 5. Effect of **11b**, **11c** and **11d** concentration on the proliferation of *Trypanosoma cruzi* epimastigotes. The parasites were grown at 28 °C for 4 days with different concentrations (2.5–15 μM) of tested compounds. Growth rate was evaluated by counting the parasites using a Neubauer chamber. p values < 0.05 (*) and < 0.01 (**) were considered significant.

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 IC$_{50}$ 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 μM no cytotoxic effect was observed, while for Bnz was of 85 ± 5 μM.

2.5. Hemin interaction

Trypanosomatids protozoa show a strict requirement of heme-

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

Comp		IC$_{50}$ (μM)			Cytotoxicity
	Lm pro	*Tc epi*	*Tc* trypo	*Tc* amas	CC$_{50}$ (μM)
10a	> 200	> 15	≫500	≫50	
10b	75.6 ± 1.0	> 15	811.02 ± 19.35	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 ± 12.24	22.8 ± 0.9	> 100
11c	41.9 ± 0.8	11.29 ± 0.63	616.02 ± 15.80	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	–	–		–

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.

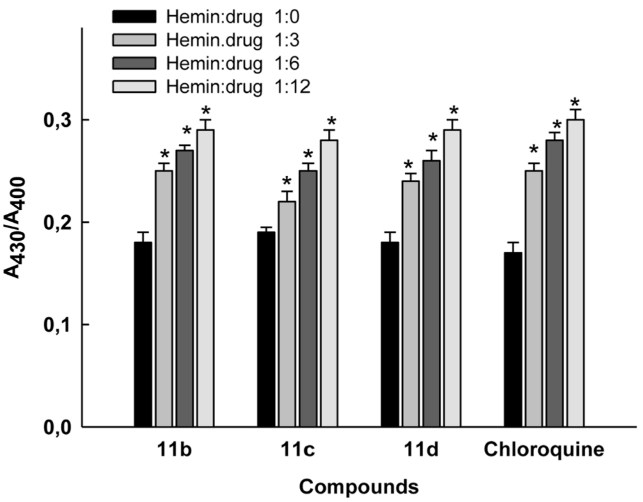


Fig. 6. Interaction of quinoline derivatives **11b–d** with hemin. After interacting the hemin (7.5 µM) with different concentrations of **11b–d** quinolines or chloroquine (22.5–90 µM), the absorption spectra of hemin was recorded. The absorbance ratio at 430 and 400 nm (A430/A400) was used to quantify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

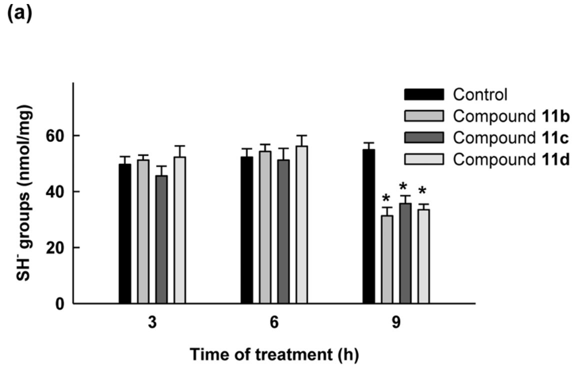
compounds such as hemoglobin, hematin or hemin during their growth [30]. This nutritional characteristic is due to its inability to synthesize heme. Since high levels of hemin and related compounds have an important cytotoxic effect mediated by ROS generation, trypanosomatids present different pathways 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.

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 µM) 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 h, 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 Fig. 7B the behavior of the three quinolines was similar, an increase of about 4 times in the fluorescence intensity of the H_2DCFDA-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.



(b)

Time of treatment (h)	Ratio (Gm_t/Gm_c)		
	11b	11c	11d
3	1.10 ± 0.22	1.20 ± 0.35	0.97 ± 0.16
6	0.95 ± 0.25	1.02 ± 0.12	1.08 ± 0.35
9	4.20 ± 0.48**	3.87 ± 0.18**	4.08 ± 0.59**

Fig. 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 µM **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 H_2DCFDA. 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 synthesized 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 4 Å 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 $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.04 M), $Ce(SO_4)2$ (0.003 M) in concentrated H_2SO_4 (10%). ^1H NMR and ^{13}C NMR spectra were recorded at room temperature in $CDCl_3$ as solvent using a Bruker AM-500 NMR instrument operating at 500.14 MHz and 125.76 MHz for ^1H and ^{13}C respectively. The ^1H NMR spectra are referenced with respect to the residual $CHCl_3$ proton of the solvent $CDCl_3$ at δ = 7.26 ppm. Coupling constants are reported in Hertz (Hz). ^{13}C NMR spectra were proton decoupled and are referenced to the middle peak of the solvent $CDCl_3$ at δ = 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 ± 0.02% of the theoretical values.

1. 2-(2-Fluor-phenylamino) quinoline (10a). Yield 66% of pure compound as a yellow oil. ^1H NMR ($CDCl_3$) δ 6.60 (m, 2H, H-14, H-16), 6.97 (d, J = 8.5 Hz, 1H, H-3), 7.16 (m, 2H, H-13, H-15), 7.33 (dt, J_1 = 1.2 Hz, J_2 = 8.0 Hz, 1H, 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, 1H, H-8), 7.83 (dd, J_1 = 1.1 Hz, J_2 = 8.0 Hz, 1H, H-5), 7.97 (d, J = 8.4 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) 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. $C_{15}H_{11}FN_2Na$ 261.0804. Found: $C_{15}H_{11}FN_2Na$ 261.0812.

2. 2-(3-Fluor-phenylamino) quinoline (10b). Yield 70% of pure compound as a light yellow oil. ^1H NMR ($CDCl_3$) 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, 2H, H-15, H-16), 7.35 (dt, J_1 = 1.1 Hz, J_2 = 8.0 Hz, 1H, H-6), 7.62 (m, 2H, H-7, H-12), 7.67 (dd, J_1 = 1.3 Hz, J_2 = 7.9 Hz, 1H, H-8), 7.84 (dd, J_1 = 1.2 Hz, J_2 = 8.0 Hz, 1H, H-5), 7.98 (d, J = 8.6 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) 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. $C_{15}H_{11}FN_2Na$ 261.0804. Found: $C_{15}H_{11}FN_2Na$ 261.0799.

3. 2-(4-Fluor-phenylamino) quinoline (10c). Yield 75% of pure compound as a yellow oil. ^1H NMR ($CDCl_3$) δ 6.89 (d, J = 8.2 Hz, 1H, H-3), 7.06 (d, J = 8.6 Hz, 2H, H-13, H-15), 7.31 (dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, 1H, H-6), 7.55 (d, J = 8.7 Hz, 2H, H-12, H-16), 7.60 (dt, J_1 = 1.5 Hz, J_2 = 7.5 Hz, 1H, H-7), 7.64 (dd, J_1 = 1.0 Hz, J_2 = 7.8 Hz, 1H, H-8), 7.77 (dd, J_1 = 1.0 Hz, J_2 = 7.6 Hz, 1H, H-5), 7.92 (d, J = 8.0 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) 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. $C_{15}H_{11}FN_2Na$ 261.0804. Found: $C_{15}H_{11}FN_2Na$ 261.0810.

4. 2-(4-Chloro-phenylamino) quinoline (10d). Yield 72% of pure compound as a light yellow oil. ^1H NMR ($CDCl_3$) δ 6.94 (d, J = 9.0 Hz, 1H, H-3), 7.32–7.33 (m, 3H, H-6, H-13, H-15), 7.52 (d, J = 7.8 Hz, 2H, H-12, H-16), 7.61 (dt, J_1 = 1.5 Hz, J_2 = 7.1 Hz, 1H, H-7), 7.66 (dd, J_1 = 1.5 Hz, J_2 = 8.1 Hz, 1H, H-8), 7.79 (dd, J_1 = 1.3 Hz, J_2 = 8.4 Hz, 1H, H-5), 7.95 (d, J = 8.9 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) 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. $C_{15}H_{11}ClN_2Na$ 277.0508. Found: $C_{15}H_{11}ClN_2Na$ 277.0512.

5. 2-(4-Carboxy-phenylamino) quinoline (10e). Yield 45% of pure compound as a light yellow oil. ^1H NMR ($CDCl_3$) δ 6.88 (d, J = 8.0 Hz, 1H, H-3), 7.30 (dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, 1H, H-6), 7.59 (dt, J_1 = 1.4 Hz, J_2 = 7.6 Hz, 1H, H-7), 7.64 (dd, J_1 = 1.2 Hz, J_2 = 7.7 Hz, 1H, H-8), 7.70 (d, J = 8.6 Hz, 2H, H-12, H-16), 7.77 (dd, J_1 = 1.1 Hz, J_2 = 7.6 Hz, 1H, H-5), 7.92 (d, J = 7.9 Hz, 1H, H-4), 8.00 (d, J = 8.6 Hz, 2H, H-13, H-15); ^{13}C NMR ($CDCl_3$) 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 (–CO_2H); HRMS: $[M+Na]^+$ Calcd. $C_{16}H_{12}N_2NaO_2$ 287.0796. Found: $C_{16}H_{12}N_2NaO_2$ 287.0790.

6. 2-(4-Vinyl-phenylamino) quinoline (10f). Yield 68% of pure compound as a light orange oil. ^1H NMR ($CDCl_3$) 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-18b), 6.67 (dd, J_1 = 10.9 Hz, J_2 = 17.5 Hz, 1H, H-17), 6.91 (d, J = 8.9 Hz, 1H, H-3), 7.30 (m, 3H, H-6, H-13, H-15), 7.50 (d, J = 7.9 Hz, 2H, H-12, H-16), 7.58 (dt, J_1 = 1.6 Hz, J_2 = 7.0 Hz, 1H, H-7), 7.63 (dd, J_1 = 1.5 Hz, 2 = 8.0 Hz, 1H, H-8), 7.77 (dd, J_1 = 1.2 Hz, J_2 = 8.5 Hz, 1H, H-5), 7.93 (d, J = 9.0 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$) 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. $C_{17}H_{14}N_2Na$ 269.1055. Found: $C_{17}H_{14}N_2Na$ 269.1053.

7. 3-(2-Fluor-phenylamino) quinoline (11a). Yield 70% of pure compound as a yellow oil. ^1H NMR ($CDCl_3$) 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, 1H, 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, 1H, H-4), 7.60 (dd, J_1 = 1.1 Hz, J_2 = 8.0 Hz, 1H, H-5), 7.98 (d, J = 8.4 Hz, 1H, H-8), 8.64 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$) 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. $C_{15}H_{11}FN_2Na$ 261.0804. Found: $C_{15}H_{11}FN_2Na$ 261.0809.

8. 3-(3-Fluor-phenylamino) quinoline (11b). Yield 80% of pure compound as a yellow oil. ^1H NMR ($CDCl_3$) δ 7.07 (ddd, J_1 = 2.1 Hz, J_2 = 8.2 Hz, 1H, H-14), 7.16 (m, 2H, H-15, H-16), 7.37 (dt, J_1 = 1.0 Hz, J_2 = 8.2 Hz, 1H, H-12), 7.47 (dt, J_1 = 1.2 Hz, J_2 = 8.0 Hz, 1H, 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, 1H, H-4), 7.61 (dd, J_1 = 1.0 Hz, J_2 = 8.0 Hz, 1H, H-5), 7.99 (d, J = 8.3 Hz, 1H, H-8), 8.65 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$) 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. $C_{15}H_{11}FN_2Na$ 261.0804. Found: $C_{15}H_{11}FN_2Na$ 261.0810.

9. 3-(4-Fluor-phenylamino) quinoline (11c). Yield 85% of pure compound as light yellow colorless oil. ^1H NMR ($CDCl_3$) 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, 2H, H-12, H-16), 7.45 (dt, J_1 = 1.3 Hz, J_2 = 7.8 Hz, 1H, H-7), 7.51 (dt, J_1 = 1.4 Hz, J_2 = 6.7 Hz, 1H, H-6), 7.57 (d, J = 2.6 Hz, 1H, H-4), 7.61 (dd, J_1 = 1.5 Hz, J_2 = 8.0 Hz, 1H, H-5), 7.99 (d, J = 8.2 Hz, 1H, H-8), 8.65 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$) 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. $C_{15}H_{11}FN_2Na$ 261.0804. Found: $C_{15}H_{11}FN_2Na$ 261.0808.

10. 3-(4-Chloro-phenylamino) quinoline (11d). Yield 82% of pure compound as a light yellow oil. ^1H NMR ($CDCl_3$) 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, 2H, H-12, H-16), 7.49 (dt, J_1 = 1.6 Hz, J_2 = 7.0 Hz, 1H, H-7), 7.51 (dt, J_1 = 1.6 Hz, J_2 = 6.9 Hz, 1H, H-6), 7.66 (dd, J_1 = 1.8 Hz, J_2 = 8.2 Hz, 1H, H-5), 7.69 (d, J = 2.7 Hz, 1H, H-4), 8.01 (d, J = 8.3 Hz, 1H, H-8), 8.69 (d, J = 2.7 Hz, 1H, H-2); ^{13}C NMR ($CDCl_3$) 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. $C_{15}H_{11}ClN_2Na$ 277.0508. Found: $C_{15}H_{11}ClN_2Na$ 277.0505.

11. 3-(4-Carboxy-phenylamino) quinoline (11e). Yield 50% of pure compound as a yellow oil. ^1H NMR ($CDCl_3$) δ 6.00 (ws, 1H, NH), 7.47 (dt, J_1 = 1.6 Hz, J_2 = 7.0 Hz, 1H, H-7), 7.49 (dt, J_1 = 1.6 Hz, J_2 = 6.9 Hz, 1H, H-6), 7.63 (dd, J_1 = 1.8 Hz, J_2 = 8.2 Hz, 1H, H-5), 7.67 (d, J = 2.7 Hz, 1H, H-4), 7.75 (d, J = 8.7 Hz, 2H, H-13, H-15), 7.91 (d, J = 8.7 Hz, 2H, H-12, H-16), 8.00 (d, J = 8.3 Hz, 1H, H-8), 8.67 (d, J = 2.7 Hz, 1H, H-2); ^{13}C NMR ($CDCl_3$) 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 (–CO_2H); HRMS: $[M+Na]^+$ Calcd. $C_{16}H_{12}N_2NaO_2$ 287.0796. Found: $C_{16}H_{12}N_2NaO_2$ 287.0801.

12. 3-(4-Vinyl-phenylamino) quinoline (11f). Yield 75% of pure compound as a dark yellow oil. ^{1}H NMR ($CDCl_3$) 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, 2H, H-6, H-12, H-16), 7.38 (d, J = 8.5 Hz, 2H, H-13, H-15), 7.46 (dt, J_1 = 1.6 Hz, J_2 = 6.8 Hz, 1H, H-7), 7.51 (dt, J_1 = 1.6 Hz, J_2 = 6.8 Hz, 1H, H-6), 7.62 (dd, J_1 = 1.6 Hz, J_2 = 8.0 Hz, 1H, 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, 1H, H-8), 8.68 (d, J = 2.7 Hz, 1H, H-2); ^{13}C NMR ($CDCl_3$) 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. $C_{17}H_{14}N_2Na$ 269.1055. Found: $C_{17}H_{14}N_2Na$ 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 μg mL^{-1}), hemin (20 μg 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 μg 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 μg 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 × 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 μg/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 IC_{50} (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 × 10^7 parasites/ml were cultured in the presence of 2.5–15 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: $\%I = \{1 - [(CD_{4t} - CD_0)/(CD_{4c} - CD_0)]\} \times 100$, where the different CDs represent the cellular density of: CD_{4t}, treated parasites on day 4; CD_0, 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 × 10^6 parasites/ml) were seeded (150 μl/well) in duplicate in a 96-well plate and treated with different concentrations of each compound (0.45–450 μM) or Bnz (0.38–380 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 β-galactosidase gene, using a parasite: cell ratio of 10:1. Then 24 h, the cultures were washed and different concentration of each compound (2–50 μM) 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 $\% I = \{1-[(Ait-Anit)/(Aic-Anic)]\} \times 100$, where Ait represents the mean A570 value recorded for Ait, treated infected cells; Anit, treated noninfected cells; Aic, untreated infected cells; and Anic, untreated noninfected cells. To calculate the IC_{50} (50% inhibitory concentration) for each parasite form, I% or %L values were plotted against the logarithm (log) of drug concentration (μM) 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% CO_2. Cells, 9 × 10^5 cell/mL were seeded in a 24-well plate and then 24 h different concentrations of compounds (5–100 μM) or Bnz (5–200 μM) were added. After 48 h of incubation, cells were washed twice with PBS and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) was added at a final concentration of 0.5 mg/ml. Plates were incubated for 1 h at 37 °C. Finally, blue precipitates were dissolved in 0.5 mL DMSO and were read on a plate reader (Spectra Count™ 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 CC_{50} (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 μM hemin and different concentrations of the compounds to be tested (22.5–90 μM). The absorption spectra of hemin was measured using a Hewlett-Packard 8452-Diode Array spectrophotometer. The absorbance ratio at 430 and

400 nm (A_{430}/A_{400}) 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 µM) during 3, 6 or 9 h. Treated-parasites were harvested by centrifugation at 12,000*g* 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 SH-groups 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 oxidant-sensitive fluorescent probe H_2DCFDA. *T. cruzi* epimastigotes growing in logarithmic phase were incubated with **11b–d** (35 µM) during 3, 6 or 9 h. Treated-parasites were harvested and stained for 45 min in the dark with 10 µM H_2DCFDA at 37 °C. As positive control, parasites were treated with 0.2 mM H_2O_2. 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 ± standard errors of the mean (SEMs). The significance of differences was evaluated using Studenťs *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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.10.053.

References

[1] J.A. Urbina, Specific chemotherapy of Chagas disease: relevance, current limitations and new approaches, Acta Trop. 115 (1) (2010) 55–68.

[2] W. Second, Report on Neglected Tropical Disease, Sustaining the Drive to Overcome the Global Impact of Neglected Tropical Disease, (2013).

[3] G.E.G. Linares, E.L. Ravaschino, J.B. Rodriguez, Progresses in the field of drug design to combat tropical protozoan parasitic diseases, Curr. Med. Chem. 13 (3) (2006) 335–360.

[4] W. De Souza, Cell biology of Trypanosoma cruzi, Int. Rev. Cytol. 86 (1984) 197–283.

[5] J. Urbina, New insights in Chagas disease treatment, Drugs Future 35 (5) (2010) 409–419.

[6] M.A. Shikanai-Yasuda, N.B. Carvalho, Oral transmission of chagas disease, Clin. Infect. Dis. 54 (6) (2012) 845–852.

[7] M.C. Chung, E.I. Ferreira, J.L. Santos, J. Giarolla, D.G. Rando, A.E. Almeida, P.L. Bosquesi, R.F. Menegon, L. Blau, Prodrugs for the treatment of neglected diseases, Molecules 13 (3) (2008) 616–677.

[8] W.H. Organization, Research Priorities for Chagas disease, Human African Trypanosomiasis and Leishmaniasis, World Health Organization technical report series, 2012, 975 v.

[9] A. Dostálová, P. Volf, Leishmania development in sand flies: parasite-vector interactions overview, Parasites Vectors 5 (1) (2012) 276.

[10] S.L. Croft, V. Yardley, Chemotherapy of leishmaniasis, Curr. Pharm. Des. 8 (4) (2002) 319–342.

[11] P.D. Marsden, T.C. Jones, Clinical manifestations, diagnosis and treatment of leishmaniasis, Leishmaniasis. Elsevier Science Publishers, Amsterdam, The Netherlands, 1985, pp. 183–198.

[12] S. Hirst, L. Stapley, The dawn of a new millennium, Parasitology Today 16 (1) (2000).

[13] E. Diro, K. Ritmeijer, M. Boelaert, F. Alves, R. Mohammed, C. Abongomera, R. Ravinetto, M. De Crop, H. Fikre, C. Adera, Use of pentamidine as secondary prophylaxis to prevent visceral leishmaniasis relapse in HIV infected patients, the first twelve months of a prospective cohort study, PLoS Negl Trop Dis 9 (10) (2015) e0004087.

[14] P. Desjeux, J. Alvar, Leishmania/HIV co-infections: epidemiology in Europe, Ann. Trop. Med. Parasitol. 97 (sup1) (2003) 3–15.

[15] R. Docampo, S.N.J. Moreno, Biochemistry of *Trypanosoma cruzi*, Am. Trypanosomiasis (2010) 365–392.

[16] J.A. Urbina, Chemotherapy of Chagas disease, Curr. Pharm. Des. 8 (4) (2002) 287–295.

[17] R.C. Zauli-Nascimento, D.C. Miguel, J.K. Yokoyama-Yasunaka, L.I. Pereira, M.A. Pelli de Oliveira, F. Ribeiro-Dias, M.L. Dorta, S.R. Uliana, In vitro sensitivity of Leishmania (Viannia) braziliensis and Leishmania (Leishmania) amazonensis Brazilian isolates to meglumine antimoniate and amphotericin B, Trop. Med. Int. Health 15 (1) (2010) 68–76.

[18] S.L. Croft, S. Sundar, A.H. Fairlamb, Drug resistance in leishmaniasis, Clin. Microbiol. Rev. 19 (1) (2006) 111–126.

[19] J. Chakravarty, S. Sundar, Drug resistance in leishmaniasis, J. Glob. Infect. dis. 2 (2) (2010) 167.

[20] S.L. Croft, M.P. Barrett, J.A. Urbina, Chemotherapy of trypanosomiases and leishmaniasis, Trends Parasitol. 21 (11) (2005) 508–512.

[21] J.A. Urbina, R. Docampo, Specific chemotherapy of Chagas disease: controversies and advances, Trends Parasit. 19 (11) (2003) 495–501.

[22] M. Ouellette, J. Drummelsmith, B. Papadopoulou, Leishmaniasis: drugs in the clinic, resistance and new developments, Drug Resist. Updates 7 (4) (2004) 257–266.

[23] H. Sindermann, J. Engel, Development of miltefosine as an oral treatment for leishmaniasis, Trans. R. Soc. Trop. Med. Hyg. 100 (Supplement 1) (2006) S17–S20.

[24] T.P. Dorlo, M. Balasegaram, J.H. Beijnen, P.J. de Vries, Miltefosine: a review of its pharmacology and therapeutic efficacy in the treatment of leishmaniasis, J. Antimicrob. Chemother. 67 (11) (2012) 2576–2597.

[25] N. Ganguly, Oral miltefosine may revolutionize treatment of visceral leishmaniasis, TDR News 68 (2) (2002).

[26] F.J. Pérez-Victoria, S. Castanys, F. Gamarro, Leishmania donovani resistance to miltefosine involves a defective inward translocation of the drug, Antimicrob. Agents Chemother. 47 (8) (2003) 2397–2403.

[27] L. Carvalho, J.R. Luque-Ortega, C. López-Martín, S. Castanys, L. Rivas, F. Gamarro, The 8-aminoquinoline analogue sitamaquine causes oxidative stress in Leishmania

donovani promastigotes by targeting succinate dehydrogenase, Antimicrob. Agents Chemother. 55 (9) (2011) 4204–4210.

[28] P. Loiseau, S. Cojean, J. Schrével, Sitamaquine as a putative antileishmanial drug candidate: from the mechanism of action to the risk of drug resistance, Parasite 18 (2) (2011) 115–119.

[29] M.M. Fernández, E.L. Malchiodi, I.D. Algranati, Differential effects of paromomycin on ribosomes of Leishmania mexicana and mammalian cells, Antimicrob. Agents Chemother. 55 (1) (2011) 86–93.

[30] J.A. Urbina, Recent clinical trials for the etiological treatment of chronic Chagas disease: advances, challenges and perspectives, J. Eukaryot. Microbiol. 62 (1) (2015) 149–156.

[31] G.C. Lechuga, J.C. Borges, C.M. Calvet, H.P. de Araújo, A.A. Zuma, S.B. do Nascimento, M.C.M. Motta, A.M.R. Bernardino, M.C. de Souza Pereira, S.C. Bourguignon, Interactions between 4-aminoquinoline and heme: promising mechanism against Trypanosoma cruzi, Int. J. Parasitol.: Drugs Drug Resistance 6 (3) (2016) 154–164.

[32] K. Krafts, E. Hempelmann, A. Skórska-Stania, From methylene blue to chloroquine: a brief review of the development of an antimalarial therapy, Parasitol. Res. 111 (1) (2012) 1–6.

[33] V. Srivastava, H. Lee, Chloroquine-based hybrid molecules as promising novel chemotherapeutic agents, Eur. J. Pharmacol. 762 (2015) 472–486.

[34] Y. Li, C. de Kock, P.J. Smith, H. Guzgay, D.T. Hendricks, K. Naran, V. Mizrahi, D.F. Warner, K. Chibale, G.S. Smith, Synthesis, characterization, and pharmacological evaluation of silicon-containing aminoquinoline organometallic complexes as antiplasmodial, antitumor, and antimycobacterial agents, Organometallics 32 (1) (2012) 141–150.

[35] K. Abouzid, S. Shouman, Design, synthesis and in vitro antitumor activity of 4-aminoquinoline and 4-aminoquinazoline derivatives targeting EGFR tyrosine kinase, Bioorg. Med. Chem. 16 (16) (2008) 7543–7551.

[36] A.T. Taher, N.A. Khalil, E.M. Ahmed, Y.M. Ragab, Synthesis of certain 2-substituted-1H-benzimidazole derivatives as antimicrobial and cytotoxic agents, Chem. Pharm. Bull. 60 (6) (2012) 778–784.

[37] A. Fournet, R. Hocquemiller, F. Roblot, A. Cavé, P. Richomme, J. Bruneton, Les Chimanines, nouvelles quinoleines substituees en 2, isolees d'une plante bolivienne antiparasitaire: Galipea longiflora, J. Nat. Prod. 56 (1993) 1547 1547.

[38] M.A. Fakhfakh, A. Fournet, E. Prina, J.-F. Mouscadet, X. Franck, R. Hocquemiller, B. Figadère, Synthesis and biological evaluation of substituted quinolines: potential treatment of protozoal and retroviral co-infections, Bioorg. Med. Chem. 11 (23) (2003) 5013–5023.

[39] J.M. Kraus, C.L. Verlinde, M. Karimi, G.I. Lepesheva, M.H. Gelb, F.S. Buckner, Rational modification of a candidate cancer drug for use against Chagas disease, J. Med. Chem. 52 (6) (2009) 1639.

[40] J.M. Kraus, H.B. Tatipaka, S.A. McGuffin, N.K. Chennamaneni, M. Karimi, J. Arif, C.L. Verlinde, F.S. Buckner, M.H. Gelb, Second generation analogs of the cancer drug clinical candidate tipifarnib for anti-Chagas disease drug discovery, J. Med. Chem. 53 (10) (2010) 3887.

[41] V.S. Gopinath, J. Pinjari, R.T. Dere, A. Verma, P. Vishwakarma, R. Shivahare, M. Moger, P.S.K. Goud, V. Ramanathan, P. Bose, Design, synthesis and biological evaluation of 2-substituted quinolines as potential antileishmanial agents, Eur. J. Med. Chem. 69 (2013) 527–536.

[42] A.F. Slater, Chloroquine: mechanism of drug action and resistance in Plasmodium falciparum, Pharmacol. Ther. 57 (2–3) (1993) 203–235.

[43] S. Pagola, P.W. Stephens, D.S. Bohle, A.D. Kosar, S.K. Madsen, The structure of malaria pigment β-haematin, Nature 404 (6775) (2000) 307–310.

[44] D.J. Sullivan, H. Matile, R.G. Ridley, D.E. Goldberg, A common mechanism for blockade of heme polymerization by antimalarial quinolines, J. Biol. Chem. 273 (47) (1998) 31103–31107.

[45] J.M. Combrinck, T.E. Mabotha, K.K. Ncokazi, M.A. Ambele, D. Taylor, P.J. Smith, H.C. Hoppe, T.J. Egan, Insights into the role of heme in the mechanism of action of antimalarials, ACS Chem. Biol. 8 (1) (2013) 133.

[46] R. Thomé, S.C.P. Lopes, F.T.M. Costa, L. Verinaud, Chloroquine: modes of action of an undervalued drug, Immunol. Lett. 153 (1) (2013) 50–57.

[47] G. García Liñares, G. Parraud, C. Labriola, A. Baldessari, Chemoenzymatic synthesis and biological evaluation of 2- and 3-hydroxypyridine derivatives against Leishmania mexicana, Bioorg. Med. Chem. 20 (15) (2012) 4614–4624.

[48] M.F. Abdel-Megeed, B.E. Badr, M.M. Azaam, G.A. El-Hiti, Synthesis, antimicrobial and anticancer activities of a novel series of diphenyl 1-(pyridin-3-yl) ethylphosphonates, Bioorg. Med. Chem. 20 (7) (2012) 2252–2258.

[49] P. Kassis, J. Brzeszcz, V. Bénéteau, O. Lozach, L. Meijer, R. Le Guével, C. Guillouzo, K. Lewiński, S. Bourg, L. Colliandre, Synthesis and biological evaluation of new 3-(6-hydroxyindol-2-yl)-5-(Phenyl) pyridine or pyrazine V-Shaped molecules as kinase inhibitors and cytotoxic agents, Eur. J. Med. Chem. 46 (11) (2011) 5416–5434.

[50] D. Pez, I. Leal, F. Zuccotto, C. Boussard, R. Brun, S.L. Croft, V. Yardley, L.M.R. Perez, D.G. Pacanowska, I.H. Gilbert, 2, 4-Diaminopyrimidines as inhibitors of leishmanial and trypanosomal dihydrofolate reductase, Bioorg. Med. Chem. 11 (22) (2003) 4693–4711.

[51] D.P. Iwaniuk, E.D. Whetmore, N. Rosa, K. Ekoue-Kovi, J. Alumasa, A.C. de Dios, P.D. Roepe, C. Wolf, Synthesis and antimalarial activity of new chloroquine analogues carrying a multifunctional linear side chain, Bioorg. Med. Chem. 17 (18) (2009) 6560–6566.

[52] P.A. Stocks, K.J. Raynes, P.G. Bray, B.K. Park, P.M. O'Neill, S.A. Ward, Novel short chain chloroquine analogues retain activity against chloroquine resistant K1 Plasmodium falciparum, J. Med. Chem. 45 (23) (2002) 4975–4983.

[53] E. Vitaku, D.T. Smith, J.T. Njardarson, Analysis of the structural diversity, substitution patterns, and frequency of nitrogen heterocycles among U.S. FDA approved pharmaceuticals, J. Med. Chem. 57 (24) (2014) 10257–10274.

[54] D. Bompart, J. Núñez-Durán, D. Rodríguez, V.V. Kouznetsov, C.M.M. Gómez, F. Sojo, F. Arvelo, G. Visbal, A. Alvarez, X. Serrano-Martín, Anti-leishmanial evaluation of C2-aryl quinolines: Mechanistic insight on bioenergetics and sterol biosynthetic pathway of Leishmania braziliensis, Bioorg. Med. Chem. 21 (14) (2013) 4426–4431.

[55] N.M. Shah, M.P. Patel, R.G. Patel, New N-arylamino biquinoline derivatives: synthesis, antimicrobial, antituberculosis, and antimalarial evaluation, Eur. J. Med. Chem. 54 (2012) 239–247.

[56] R. de Meneses Santos, P.R. Barros, J.H. Bortoluzzi, M.R. Meneghetti, Y.K.C. da Silva, A.E. da Silva, M. da Silva Santos, M.S. Alexandre-Moreira, Synthesis and evaluation of the anti-nociceptive and anti-inflammatory activity of 4-aminoquinoline derivatives, Bioorg. Med. Chem. 23 (15) (2015) 4390–4396.

[57] W. Liao, G. Hu, Z. Guo, D. Sun, L. Zhang, Y. Bu, Y. Li, Y. Liu, P. Gong, Design and biological evaluation of novel 4-(2-fluorophenoxy) quinoline derivatives bearing an imidazolone moiety as c-Met kinase inhibitors, Bioorg. Med. Chem. 23 (15) (2015) 4410–4422.

[58] R.S. Upadhayaya, S.S. Dixit, A. Földesi, J. Chattopadhyaya, New antiprotozoal agents: their synthesis and biological evaluations, Bioorg. Med. Chem. Lett. 23 (9) (2013) 2750–2758.

[59] P.G. Quintana, G. García Liñares, S.N. Chanquia, R.M. Gorojod, M.L. Kotler, A. Baldessari, Improved enzymatic procedure for the synthesis of anandamide and N-fatty acylalkanolamine analogues: a combination strategy to antitumor activity, Eur. J. Org. Chem. 2016 (3) (2016) 518–528.

[60] S.N. Chanquia, N. Boscaro, L. Alche, A. Baldessari, G.G. Liñares, An efficient lipase-catalyzed synthesis of fatty acid derivatives of vanillylamine with antiherpetic activity in acyclovir-resistant strains, ChemistrySelect 2 (4) (2017) 1537–1543.

[61] M.A. Zígolo, M. Salinas, L. Alché, A. Baldessari, G.G. Liñares, Chemoenzymatic synthesis of new derivatives of glycyrrhetinic acid with antiviral activity. Molecular docking study, Bioorg. Chem. 78 (2018) 210–219.

[62] D.M. Chan, K.L. Monaco, R.-P. Wang, M.P. Winters, New N-and O-arylations with phenylboronic acids and cupric acetate, Tetrahedron Lett. 39 (19) (1998) 2933–2936.

[63] D.A. Evans, J.L. Katz, T.R. West, Synthesis of diaryl ethers through the copper-promoted arylation of phenols with arylboronic acids. An expedient synthesis of thyroxine, Tetrahedron Lett. 39 (19) (1998) 2937–2940.

[64] P.Y. Lam, C.G. Clark, S. Saubern, J. Adams, M.P. Winters, D.M. Chan, A. Combs, New aryl/heteroaryl C N bond cross-coupling reactions via arylboronic acid/cupric acetate arylation, Tetrahedron Lett. 39 (19) (1998) 2941–2944.

[65] G.G. Liñares, S. Gismondi, N.O. Codesido, S.N. Moreno, R. Docampo, J.B. Rodriguez, Fluorine-containing aryloxyethyl thiocyanate derivatives are potent inhibitors of Trypanosoma cruzi and Toxoplasma gondii proliferation, Bioorg. Med. Chem. Lett. 17 (18) (2007) 5068–5071.

[66] A. Ciccarelli, L. Araujo, A. Batlle, E. Lombardo, Effect of haemin on growth, protein content and the antioxidant defence system in Trypanosoma cruzi, Parasitology 134 (7) (2007) 959–965.

[67] A.B. Ciccarelli, F.M. Frank, V. Puente, E.L. Malchiodi, A. Batlle, M.E. Lombardo, Antiparasitic effect of vitamin B12 on Trypanosoma cruzi, Antimicrob. Agents Chemother. 56 (10) (2012) 5315–5320.

[68] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1) (1951) 265–275.